VOLUME XLVIII NUMBER 3

BOTANICAL GAZETTE

SEPTEMBER 1909

PRELIMINARY ACCOUNT OF THE OVULE, GAMETOPHYTES, AND EMBRYO OF *WIDDRINGTONIA CUPRESSOIDES*

W. T. SAXTON

(WITH PLATE XI AND THREE FIGURES)

INTRODUCTION

The genus Widdringtonia contains about six species of trees in equatorial and south Africa and Madagascar. A plant from tropical Africa figured by GOEBEL (**16**) and by EICHLER in ENGLER and PRANTL (**12**) as *Callitris quadrivalvis* is probably also very closely related to Widdringtonia, but differs in the much smaller number of ovules and also to some extent in the foliage, and was separated from that genus by MASTERS (**26**) as *Tetraclinis articulata*. I have not had an opportunity to examine this plant.

The genus Callitris has unfortunately been repeatedly confused with Widdringtonia, and the species of the latter are included under the former genus by BENTHAM and HOOKER (**2**), EICHLER (**12**), JACKSON (**17**), BOLUS and WOLLEY-DOD (**3**), and MARLOTH (**25**). Widdringtonia was clearly distinguished as a genus, however, by ENDLICHER (**13**), and is recognized by such authorities as MASTERS (**26, 27**) and RENDLE (**30**), as well as by SIM (**34**) and MAIDEN (**24**), the chief authorities on the forest floras of South Africa and Australia respectively. The two genera differ widely in cones and foliage, and while Widdringtonia (excluding fossil species) is restricted to south and central Africa and Madagascar, Callitris is as rigidly restricted to the Australian region. I have been fortunate in having a good opportunity to study several species of both genera growing in the Government Plantations at Tokai, the majority of the trees

coning freely, and am confident that no one who took advantage of a similar opportunity would still wish to unite the two genera.

Only two species of conifers occur native in the Cape Peninsula, and both are confined to the tops and upper slopes of the mountains; these are *Podocarpus Thunbergii* Hook. and *Widdringtonia cupressoides* Endl. (**3**). The development of Podocarpus is fairly well known, having been studied in other species (COKER **6**, BURLINGAME **4**), but that of Widdringtonia (excluding Tetraclinis) is entirely unknown so far as I am aware.

In attempting to work out the life-history the chief difficulty has been the collection of the material. The plant occurs only at an altitude of about 2000 feet and upward, and it has always required at least four or five hours to obtain a single collection. Furthermore, a considerable portion of the cones contain only abortive ovules, especially in certain localities, and this has often made the collection of even a small number of ovules very tedious. As a consequence it has only been possible to make collections at rather long intervals, and these have each only included a small number of ovules, particularly in the later stages. As these difficulties will again be encountered in trying to fill in the gaps in the present account, it is thought best to publish the results now presented. Here and there comparisons are made with Callitris, the development of which is very similar. I hope later to be able to study this genus more in detail.

I am glad to take this opportunity of thanking the authorities at Tokai for permission to collect cones of all species of Widdringtonia and Callitris grown in the Government Plantations, and also my friend Mr. E. P. PHILLIPS, for kindly collecting and fixing material on three occasions.

METHODS

The material has in almost all cases been fixed in the field, and all figures are drawn from such material. Various fixing agents have been tried, including different strengths of chromacetic acid with and without osmic acid, but the following has been found the most generally useful (CHAMBERLAIN **5**, p. 20): picric acid (saturated solution in 50 per cent. alcohol), 100^{cc}; glacial acetic acid, 5^{cc};

corrosive sublimate, 5^{gm}.[1] Material was fixed for 24 hours and then washed in 50 per cent. alcohol until the alcohol was no longer colored yellow (a week or more as a rule), then 12–24 hours in 75 per cent., 85 per cent., and 94 per cent. alcohol, and at least 60 hours in absolute alcohol changed three or more times. Both xylol and cedar oil (in 25 per cent. grades with alcohol) were tried to precede infiltration with paraffin, but the former was entirely discarded after a few trials. Material was taken from cedar oil through 25 per cent., 50 per cent., and 75 per cent. solutions of soft paraffin in cedar oil, to pure paraffin, melting point 48° C., using tiny wire-gauze baskets, for the purpose, as described by FERGUSON (**14**), and allowing 48 hours in each solution. At least 48 hours longer was allowed in two fresh lots of soft paraffin (an indication of the time required here is given by the time taken to wash out the fixing agent), 12–24 hours in a mixture of soft and hard paraffin, and finally 24 hours in hard paraffin, melting point 55° C., in which it was imbedded. These very long periods in the oven were found to be absolutely necessary to insure proper infiltration of the paraffin.

In young stages the ovules were fixed whole, or when very young a small part of the tissue of the cone was cut off bearing the ovules. Later the integument becomes too hard to cut, and it was necessary to dissect out the nucellus, a process which can be carried out fairly easily in practice. In some stages after fertilization the prothallus was dissected out, and where nearly mature embryos were present these were fixed alone. The staminate cones were fixed whole, being very small, but required even longer periods in the oven than those mentioned above.

The stains were (1) Delafield's hematoxylin diluted 8–10 times, allowed to stain 4–12 hours, extracted with very dilute acid (aqueous), and followed by a thorough washing in water; and (2) Flemming's triple stain. The best results were obtained with the former, especially after the fixing agent mentioned above. It appears to be always the case that the very best staining effects of Delafield's hematoxylin are obtained after a fixing agent containing corrosive sublimate. The value of this hematoxylin as a nuclear stain has not at present been realized.

[1] I am indebted to Mr. A. J. BALLANTINE for suggesting the use of this reagent.

DESCRIPTION

The youngest ovulate cones seen were about 3 or 4^{mm} across, when the two decussate pairs of scales were widely spreading. The ovules are generally 20–30 in number and appear to be evenly distributed over the broadened end of the axis. The subsequent development proves this position to be only apparent, and that they are actually situated on the fused bases of the scales. By considerable growth of the basal part of the scales the ovules are carried farther apart, and after the former have grown together the latter are found on the sides of the ridges where the lower and upper scales meet.

The youngest ovule found is shown in *fig. 1*. The comparatively long tubular micropyle is very noticeable even at this early stage; the upper cells are already dead and empty and growth of the integument proceeds only at its base. The layer of small cells with dense contents lining the basal part of the micropyle grow actively some time after pollination and narrow considerably the micropylar opening at this part. Apparently, however, the micropyle is never completely closed by this means, but only by the accumulation of dust, etc., at its apex. The integument so soon becomes too hard to section satisfactorily that it is impossible to speak with confidence on this point.

The staminate cones are mature at about the same time that the ovules are found in this condition. They are very small and so inconspicuous until they change color at or near maturity that I have never succeeded in collecting immature cones with a view to following the development of the microspores. The sporophylls are peltate and somewhat pointed toward the apex of the cone and bear five pollen sacs abaxially placed on the stalk, the wall of the microsporangia being only one cell thick (*fig. 2*). The pollen grains have an exceptionally thick cell wall, and only a single nucleus can be distinguished in the mature grain, and in early stages of germination (*figs. 3, 4*). It seems usual in the Cupressineae for no prothallial cell to be formed in the male gametophyte, COKER (**8**) having noted their absence in eight or nine genera, including Callitris. It is of course impossible to assert their absence in Widdringtonia without obtaining preparations of microspores of various ages, but it may be assumed that none is present. As a large number of pollen grains have been

examined in the stages here figured, it seems scarcely possible that a second nucleus can be present, but it is unusual for the division giving rise to the generative and tube nuclei to be delayed so long. COKER (**8**) reports that this division occurs after shedding in certain cases, but says that it occurs before shedding in *Callitris* sp. As will be seen, however, the whole history of the male gametophyte shows a minimum of nuclear activity.

The pollen grain has a fairly thin cellulose endospore and a very thick and rather hard exospore (*fig. 4*), and on germination (as shown in the figure) the exospore alone begins to grow, without any immediate growth of the endospore or any trace of tube formation. The number of pollen grains which begin to germinate in each ovule varies from one to four, three being the most usual number (*fig. 3*). The ovule here figured closely resembles that of *fig. 1*, and therefore only a few of the cells have been drawn.

No trace of a megaspore mother cell or cells can be seen in ovules of this age, and stages to illustrate megasporogenesis are unfortunately wanting at present. GOEBEL (**16**) figures an ovule of Tetraclinis (*Callitris quadrivalvis*) in which a considerable number of what are probably megaspore mother cells are figured. Several ovules considerably older than that just mentioned show the nucellus clearly differentiated into peripheral and central regions. This central region and the innermost cells of the peripheral region are represented in *fig. 5*. The cells of the central part are somewhat larger and are characterized by having only a very scanty supply of cytoplasm. Probably these cells are a large group of megaspores, one of which later grows to form the prothallus. In this case GOEBEL'S figure of Tetraclinis would doubtless also serve to illustrate the corresponding structure in Widdringtonia.

It is certain in any case that normally only one megaspore develops, since no "secondary prothalli" have been found, as described by LAWSON (**20**) in *Sequoia sempervirens*. An ovule of *Callitris verrucosa*, however, has been sectioned, which contained two secondary prothalli (*text fig. 1*). In the next stage figured the very large prothallus is already formed. The embryo sac is lined with cytoplasm containing a single layer of free nuclei and bounding one large central vacuole. *Fig. 6* is a sketch of a whole ovule in

optical section at this stage, showing the winged integument (the wings are only one cell thick), nucellus, and prothallus. The long tubular micropyle is now very noticeable. The ovules are nearly all curved like this one, so that there is only one plane of symmetry in the ovule. Occasionally a second curvature is found at right angles to this one, in which case longitudinal sections are apt to be puzzling unless a whole series is carefully studied. Cones containing ovules of about the age shown in *fig. 6* are growing on the middle branch in the accompanying photograph (*text fig. 2*). Those on the right have just been pollinated and those on the left have young embryos, all being gathered February 28, 1909.

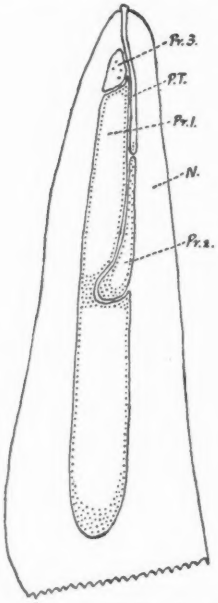

FIG. 1.—Median longitudinal section of nucellus, *n*, of *Callitris verrucosa: pt*, pollen tube; *pr* , primary prothallus; pr^2, pr^3, secondary prothalli. ×40.

Fig. 7 shows the apex of a nucellus about the same age as that in *fig. 6*. Both preparations indicate that only a single pollen tube is present and a case is only rarely met with in which more than one pollen tube develops; though, as seen above, three usually begin to germinate. Only two very small nuclei are found in the pollen tube of this age, no doubt the tube and generative nuclei.

Fig. 8 represents a very small part of the prothallus with its lining layer of cytoplasm and two of the free nuclei. In some preparations, such as the one figured, the nuclei can be seen to be paired, probably indicating that a simultaneous division has recently taken place. None of these divisions have been seen, but if, as is probable, they occur always very rapidly and simultaneously there might be only about ten separate periods of an hour or two each, extending over three months or more, during which the requisite stages could be obtained. It may also be that the time of day or night at which these divisions occur is always about the same, though there is no evidence that this is so with other divisions; at any rate

in the sporophyte, collections at 9 A. M., 12 noon, and 6 or 7 P. M. show an approximately similar number of nuclear figures.

It is noticeable in *fig. 8* that the nuclei project slightly into the vacuole. Later on the layer of cytoplasm thickens considerably and the nuclei are then completely sunk in it. *Figs. 9* and *10* indicate the principal changes met with after the last stage figured. *Fig. 9* shows clearly the process of wall formation in the prothallus,

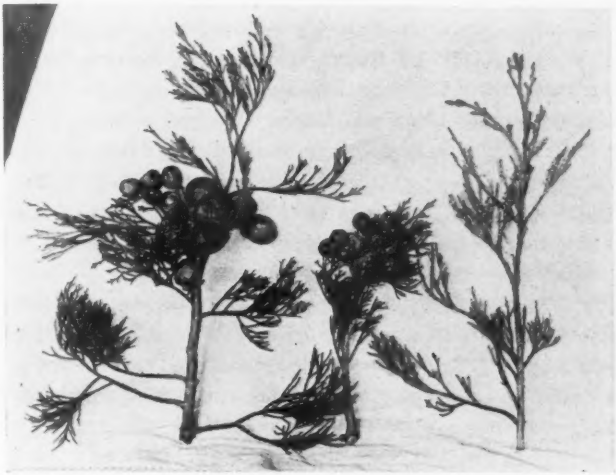

FIG. 2.—*Widdringtonia cupressoides* (see text). ×½.

alveoli being organized as described by SOKOLOWA (**35**) and other writers for various gymnosperms. The cells at first formed are invariably uninucleate, and the original cell walls persist. The binucleate and multinucleate condition met with later (see below) does not arise, therefore, in the same way as the binucleate prothallus cells of Cryptomeria described by LAWSON (**21**). The pollen tube has meanwhile reached the tip of the prothallus, and even before wall formation begins, it penetrates the megaspore membrane and grows down just inside of it, on or near the surface of the prothallus, to about one-third or one-half way down.

Fig. 10 is drawn from the same series of sections as *fig. 9* and represents the tip of the pollen tube which has in this way pene-

trated nearly half-way down the prothallus. Three nuclei are now met with in the pollen tube, all imbedded in a rather dense mass of cytoplasm. One nucleus is considerably larger than the other and is clearly differentiated from the cytoplasm. The two smaller (stalk and the tube nuclei), on the other hand, are only with difficulty distinguished either in structure or staining capacity from the surrounding cytoplasm. The larger nucleus with its cytoplasm is evidently the body cell, and its structure is shown in detail in *fig. 11*.

Fig. 12 is a sketch of a longitudinal section of the upper half of a nucellus at a slightly later stage, when cell division in the prothallus is just completed. The tube with its conspicuous body cell can be seen in the position described above. The end of this pollen tube is shown in detail in *fig. 13;* and no nucleus except that of the body cell can now be found. A careful and repeated search in this and adjoining sections of the series (which is quite complete) failed to reveal any trace of the tube and stalk nuclei, even with an oil-immersion objective. Unfortunately the tube figured was the only one showing this stage (all others were before cell division in the prothallus was complete), and the figure will probably therefore be regarded as abnormal; but the whole structure of the ovule and pollen tube seemed perfectly normal in other respects, the fixation was entirely satisfactory, as was the staining, and there was not the least indication that any part of the tube or its contents had washed off the slide during staining, etc. The staining reactions of the tube and stalk nuclei of *fig. 10*, together with their absence in *fig. 13*, indicate that they break down completely and that their substance becomes blended with the cytoplasm.

The cytological characters of the body cell nucleus are indicated in *fig. 14*, and a comparison with the nucleus in *fig. 11* suggests that division will shortly take place, giving rise to two sperm nuclei. Up to this point no trace of archegonia or archegonium initials can be seen.

Fig. 15 represents the upper half of the prothallus (and part of the nucellus) at a considerably later stage, after the archegonia are mature and fertilization has been effected.[2] The distribution of the archegonia is very remarkable and recalls that described for *Sequoia*

[2] I was unfortunately unable to make any collections for a number of weeks at this time.

sempervirens by ARNOLDI (**1**) and LAWSON (**20**). Their distribution is evidently determined largely by the position of the pollen tube. Thirty-eight archegonia are shown in this figure, but only those are indicated parts of which at least could be seen in a single section; the total number is slightly over fifty. Of several prothalli collected on the same date, one other showed an almost identical structure, but the rest were somewhat older and the archegonia were disorganized. There was an indication, however, that the number of archegonia in these other prothalli was considerably less, though their distribution must have been essentially similar.

The lowest group of archegonia from *fig. 15* has been drawn on a larger scale in *fig. 16*. Of these archegonia the lowest but one and the lowest but three have evidently been fertilized, as each contains a proembryo. This fact, together with the presence of a single pollen tube, indicates that, as is usual in Cupressineae (LAWSON **23**), two sperm cells are organized and both are functional. However, only indirect evidence is available in the present case. Unfortunately the unfertilized archegonia of the group are not in very good condition, having doubtless been organized some time previously and being about to disintegrate; hence it is quite possible that the details exhibited are to some extent different from those of a recently formed archegonium.

Only the lowest archegonium of the group has any trace of what might be interpreted as neck cells, and even this cell (shaded in the figure) is probably only a prothallus cell which happens to lie immediately over the archegonium. It is quite clear that the archegonia arise from cells deep in the prothallus, and possibly this may be the reason why no neck cells are formed, though LAWSON (**20**) records them in Sequoia; but in that genus the archegonia grow in such a way as to push their necks to the surface, whereas in Widdringtonia they remain deep-seated in the prothallus. It may be that the neck cells disintegrate entirely and leave no trace, and this would be the natural explanation in the case of fertilized archegonia, as noted by KILDAHL (**18**) in connection with a similar absence of neck cells in *Phyllocladus alpinus;* there is no apparent reason, however, to suppose that the neck cells have completely disappeared in all the unfertilized archegonia, and I believe that none are ever formed.

Scarcely any trace of jacket cells can be seen in the group of archegonia of *fig. 16*, and they are never more than very feebly organized. In this respect the condition in Cephalotaxus as described by COKER (**9**) may be compared, where jacket cells are sometimes replaced by ordinary prothallial cells; and in Torreya where COULTER and LAND (**11**) note the absence of jacket cells until after fertilization. Their absence in this genus and in Widdringtonia is probably to be correlated with the small size of the archegonia.

If the nuclear phenomena in these partially abortive archegonia are to be taken as representing normal conditions, the central nucleus divides (*fig. 16*, sixth archegonium from bottom) to form the egg and ventral nuclei (see also the top archegonium). The ventral nucleus seems usually to disappear completely, leaving only a centrally placed egg nucleus, as shown in four of the archegonia in the figure.

The lower of the two proembryos referred to above (*fig. 16*) closely resembles the proembryo of Sequoia (LAWSON **20**), but the upper shows that more cells are formed than in that genus. It is noticeable that the proembryo practically fills the archegonium in each case. The only conifers previously described in which this is the case are Torreya (COULTER and LAND **11**) and Sequoia (LAWSON **20**). This fact is likely in all three cases to be correlated with the small size of the archegonium, and is probably of no phylogenetic importance. Only one of the several cells of the proembryo forms the suspensor and one forms the embryo. The others must disintegrate rapidly, as in the next stage they are no longer recognizable (*fig. 17*).

Figs. 16, *17*, and *21* show stages in the development of the multinucleate endosperm mentioned above. Although in early stages numerous uninucleate cells may be seen, as well as a large number of binucleate cells, yet the actual origin of the binucleate condition has only been indicated by two karyokinetic figures and the remains of a spindle between the two nuclei of one cell. Sometimes two nuclei come to lie almost in contact in a cell and have thus often suggested that direct division of the nucleus has occu red, but a careful search has failed to confirm this suggestion. It seems perfectly clear, therefore, that the binucleate (and in some cells multinucleate) condition arises by karyokinetic division of the original single nucleus.

Although in *Widdringtonia cupressoides* the evidence upon which this conclusion rests is perhaps slender, yet the same phenomenon has been seen in *W. Whytei* and in two species of Callitris. In the latter abundant evidence of the origin of the binucleate condition has been obtained in both *C. cupressiforme* and *C. Muelleri*.[3] Here a considerable number of nuclei have been found in every stage of karyokinetic division from earliest prophase to fully formed binucleate cells. The four-nucleate condition is very much less common in both Widdringtonia and Callitris, and it is therefore not surprising that a second mitotic division has not been met with, but doubtless it also occurs. A very limited number of cases is found in which five nuclei are present in a single cell.

The number of chromosomes in the division just mentioned is of course the reduced one. In Callitris both the haploid and diploid chromosomes have been approximately counted, the latter being about 24 and the former almost certainly 12 in both species mentioned. In *Widdringtonia cupressoides*, in one of the two nuclear figures mentioned above, the chromosomes were just starting to separate from the equatorial plane, and in the other they had advanced about half-way to the poles. It was possible to count the group of daughter chromosomes approximately in three out of the possible four cases, and the number was about 6 in each case. The sporophytic number has been found to be about 12 (certainly not more than 14). One dividing nucleus is figured (*fig. 22*) in which 12 chromosomes seem clearly indicated; this is from a very young embryo. It is curious in two genera so closely allied as Widdringtonia and Callitris that the number of chromosomes in the one should be approximately double that found in the other, but similar differences have been noted in even more closely related plants (GATES **15**, ROSENBERG **32, 33**). So far as I am aware, no case has previously been recorded in which a multinucleate prothallus persists in a conifer, but this is certainly the case in Widdringtonia, as *fig. 21* (from quite an old prothallus) clearly shows. An entirely binucleate prothallus is present in Cryptomeria at one stage (LAWSON **21**), but subsequent cell divisions

[3] I am not quite certain that the second species is correctly named, but both cones and foliage seem to agree very closely with description and figures given by MAIDEN (**24**).

reestablish the uninucleate condition. Multinucleate jacket cells are reported by LAWSON (**23**) in various Cupressineae, also by COULTER and LAND (**11**) in Torreya, and by KILDAHL (**18**) in Phyllocladus. Apparently no case has previously been reported, however, in which a multinucleate prothallus persists in the conifers, but the record may easily have been overlooked by the writer, owing to a large proportion of the literature not being available to him. LAWSON (**23**), however, makes no mention of such a case. Parallel cases are reported among the Gnetales (LAND **19**, PEARSON **28**), but this in itself is probably of little importance, especially as the origin of such cells is different in these cases.

The development of the embryo shows no peculiarities and closely resembles the general sequence of events as described for other conifers, an apical cell being organized for a very short time (*fig. 18*); the presence of embryonal tubes is to be noted in *fig. 19*. In the mature embryo the cotyledons are two (very rarely three) in number and usually of the same length; sometimes, however, one cotyledon is conspicuously shorter than the other.

COMPARISON WITH WIDDRINGTONIA WHYTEI AND CALLITRIS

One collection of *W. Whytei*, growing in the Tokai plantations, was made on January 11, 1909, but did not yield results of much importance. It clearly agrees with *W. cupressoides* in the presence of laterally placed archegonia and in the multinucleate endosperm.

Single collections were also made of three species of Callitris (*C. verrucosa*, *C. cupressiforme*, and *C. Muelleri*)[4] during the second week in January. Of four ovules of the first-named species sectioned, one showed secondary prothalli (*text fig. 1*), and in each the prothalli were in the free nuclear condition and one pollen tube only was found, which had penetrated a short way down the side of the prothallus and contained three nearly equal nuclei (*text fig. 3*). The upper of the three, however, stains more sharply than the other two and suggests the same sequence of events as described above for Widdringtonia. The ovules of the other two species contained young embryos in different stages of development (similar to *figs. 17–19*). The unfertilized archegonia are much too disorganized to make out any-

[4] See footnote, p. 171.

thing beyond the fact that they occur in a lateral group (or possibly more than one group). The formation of the multinucleate prothallial cells has already been described. Sufficient evidence has been obtained to indicate that the development in Callitris is essentially similar to that of Widdringtonia.

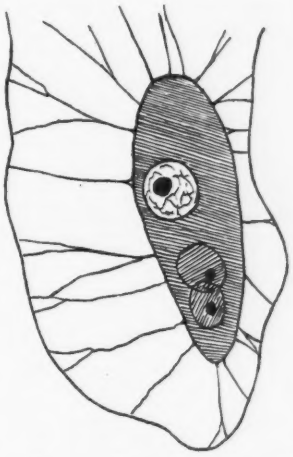

FIG. 3.—Tip of pollen tube of *Callitris verrucosa*. ×778.

DISCUSSION

With the exception of GOEBEL'S (**16**) figure of Tetraclinis (*Callitris quadrivalvis*) and COKER'S (**8**) statement about the pollen grain of Callitris, I am aware of no contribution to the knowledge of the gametophytes in the Actinostrobeae. As shown by MASTERS (**26**), there is a very close agreement between the four genera of this section of the Cupressineae in sporophyte characters, and the present investigation shows that the section is much more clearly differentiated from other Cupressineae in the gametophyte than in the sporophyte characters. Especially is this the case in the female gametophyte, where the peculiar position of the archegonia and the persistently multinucleate prothallus constitute a sharp distinction from typical Cupressineae. On the other hand, the position of the archegonia is similar to that found in Sequoia, and I hesitate to emphasize the differences in structure of the archegonia, as described above, until the development has been more closely followed. It has already been suggested that the Cupressineae have been derived from the Sequoiaceae, and it is quite possible that the Actinostrobeae constitute the connecting link between the two tribes.

It is just possible, however, that the developmental details may have a wider significance than this in indicating an approach to the conditions met with in the Gnetales. If the apparent absence of neck cells in the deep-seated archegonia is confirmed, a comparison is at once suggested with the multinucleate prothallial tubes of Tumboa

(Welwitschia).[5] A consideration of this question and also of the possible relation between the multinucleate prothallial cells of the two genera is better postponed, however, until PEARSON'S later researches on Tumboa have been published, and a closer series has been obtained in Widdringtonia.[6]

One interesting point, suggested by the fact that karyokinetic divisions occur in the prothallus at about the same time as the ventral nucleus is cut off, is that all the cells of the prothallus are potential archegonia. If the neck cells are really absent, the only essential difference between an archegonium with its egg and ventral nuclei and the other binucleate cells of the prothallus is one of size.

SUMMARY

1. The genus Widdringtonia is quite distinct from Callitris and should be kept as a distinct genus, excluding Tetraclinis.

2. A fixing agent not generally employed in cytological work has been found to give better results than chromacetic mixtures with and without osmic acid.

3. The male gametophyte is of the most reduced type yet recorded in the gymnosperms, no division of the microspore occurring until some time after pollination, and the tube and stalk nuclei disappearing before the body cell divides and before the archegonium initials can be recognized.

4. A very large number of megaspores are probably formed, but only one of these ever forms a prothallus.

5. The early development of the prothallus is perfectly normal.

6. A very large number of archegonia (over 50) are formed, and these are arranged in a number of groups near the margin of the prothallus on the side down which the pollen tube grows. They are confined to the upper half of the prothallus (but absent from the apex).

7. The archegonia arise from deep-seated cells of the prothallus and never grow through to the margin.

[5] See RENDLE'S remarks (**30**) on the correct generic name of this remarkable plant.

[6] Since the above was written, an abstract of PEARSON'S paper (**29**) has been published. He now finds the origin of the endosperm in Tumboa to be quite different from what was expected (cf. PEARSON **28**).

8. Jacket cells are either absent or sometimes very feebly developed, and apparently neck cells are entirely absent from the archegonia.

9. The central nucleus of the archegonium probably divides to form the egg nucleus and a ventral nucleus.

10. By karyokinetic divisions in the prothallus cells, about and after the time of fertilization, they become binucleate and in some cases four- or even five-nucleate.

11. The multinucleate condition persists, differing in this respect from Cryptomeria, as well as in the origin of the binucleate cells.

12. The haploid and diploid numbers of chromosomes are respectively 6 and 12 (approximately). In two species of Callitris the numbers a-e approximately 12 and 24.

13. From the occurrence of proembryos and embryos in pairs it is concluded that fertilization of two archegonia is effected by two sperms from a single pollen tube.

14. The proembryo contains eight or more cells, one of which forms a suspensor and the other the embryo. Early stages of the proembryo are wanting.

15. Embryo development is quite normal, and embryonal tubes are formed.

16. A resemblance is noted in certain points with the development of *Sequoia sempervirens*.

17. A comparison is provisionally suggested with the Gnetales, especially with the genus Tumboa.

SOUTH AFRICAN COLLEGE
CAPETOWN

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An asterisk indicates that the original paper has not been consulted.

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EXPLANATION OF PLATE XI

All figures were drawn with the aid of a Zeiss microscope, lenses, and camera lucida; *figs. 4, 8, 11, 14,* and *22* with a 2^{mm} oil-immersion objective, and the other figures with various dry objectives. Magnifications were measured directly by comparison with a stage micrometer in each case. Each figure is oriented with the long axis of cone or ovule vertical except *figs. 8* and *22*. The sections were cut out with a Cambridge rocking microtome to a thickness of 4 to 10 μ.

FIG. 1.—Median longitudinal section of very young ovule, not yet pollinated; January 7, 1909. ×180.

FIG. 2.—Median longitudinal section of staminate cone, slightly diagrammatic; only the apical three-fourths of the cone are shown; January 7, 1909. ×36.

FIG. 3.—Median longitudinal section of a pollinated ovule (drawn from two sections); January 7, 1909. ×147.

FIG. 4.—Germinating pollen grain from a similar ovule to that shown in *fig. 3;* January 7, 1909. ×725.

FIG. 5.—Median longitudinal section of central part of ovule, showing very large number of megaspores (?); May 3, 1908. ×180.

FIG. 6.—Sketch of whole ovule in optical section (cleared in cedar); oil at the apex of the nucellus is a pollen tube; June 30, 1908. ×10.

FIG. 7.—Pollen tube in apex of nucellus of similar age to that shown in *fig. 6;* May 25, 1908. ×180.

FIG. 8.—Very small part of prothallus of same ovule as *fig. 7;* vacuole to the right, membrane to the left. ×1720.

FIG. 9.—Small part of a prothallus in the process of forming cell walls; August 25, 1908. ×310.

FIG. 10.—The tip of a pollen tube which has penetrated nearly half-way down the prothallus of *fig. 9* (cut obliquely and drawn from two sections). ×310.

FIG. 11.—The body cell of *fig. 10* more highly magnified. ×725.

FIG. 12.—Upper half of median longitudinal section of nucellus after wall formation is complete in the prothallus, showing the characteristic position of the pollen tube; August 25, 1908. ×24.

FIG. 13.—Tip of pollen tube of *fig. 12*. ×310.

FIG. 14.—Body cell nucleus of *fig. 13*. ×1240.

FIG. 15.—Upper half of median longitudinal section of nucellus, *n*, and prothallus, *p*, showing position of pollen tube and of archegonia; drawn from several sections; *t*, pollen tube; January 7, 1909. ×28.

FIG. 16.—The lowest group of archegonia of *fig. 15*, two containing proembryos. ×130.

FIG. 17.—Suspensor bearing a single embryo cell at its apex; note multinucleate prothallus cells in this and previous figure; *s*, suspensor; *e*, embryo cell; January 7, 1909. ×310.

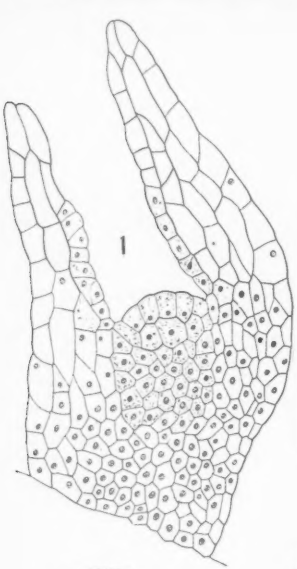
FIG. 18.—Very young embryo in median longitudinal section; March 8, 1908. ×505.

FIG. 19.—Older embryo, showing dermatogen differentiated and embryonal tubes; January 7, 1909. ×490.

FIG. 20.—Outline sketch of median longitudinal section of nearly mature embryo; March 8, 1908. ×9.

FIG. 21.—Two multinucleate cells from an old prothallus; March 8, 1908. ×540.

FIG. 22.—Dividing nucleus from an embryo between the ages of those shown in *figs. 17* and *18* respectively. ×1500.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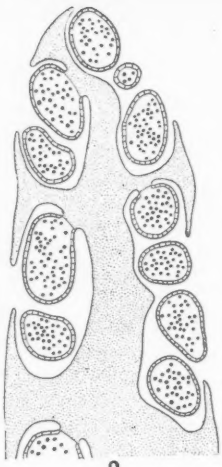
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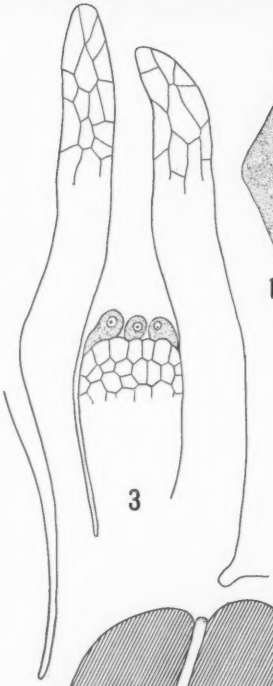
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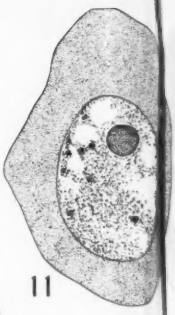
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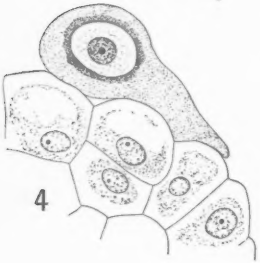
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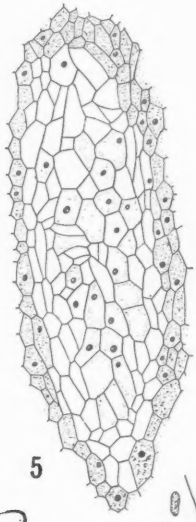
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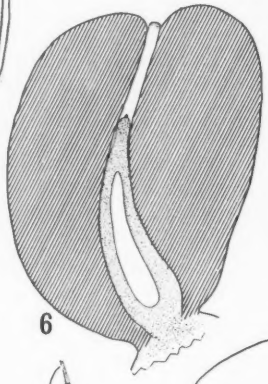
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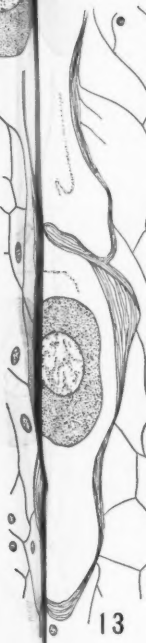
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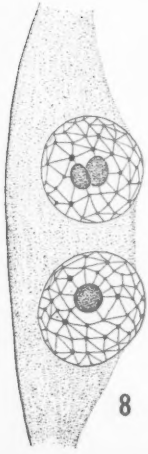
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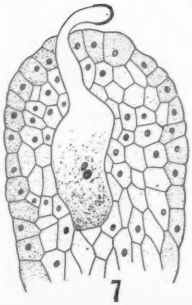
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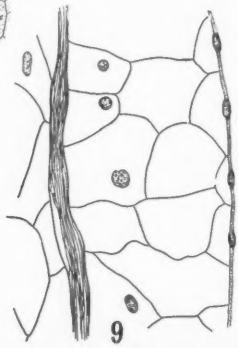
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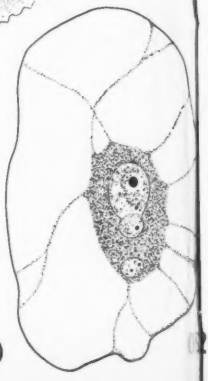
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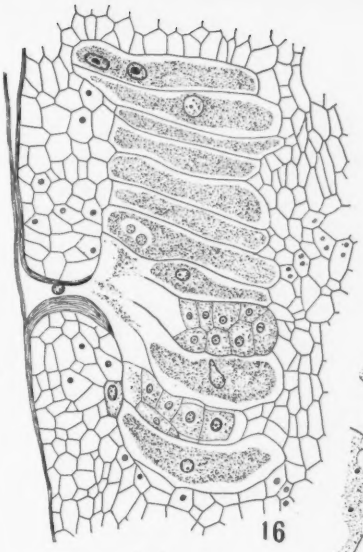

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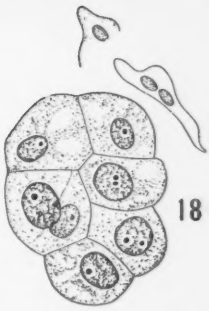

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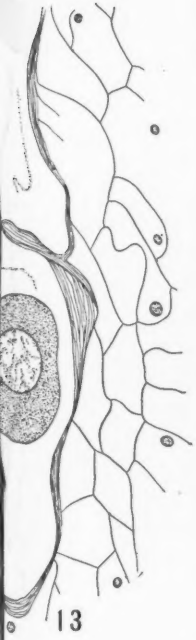

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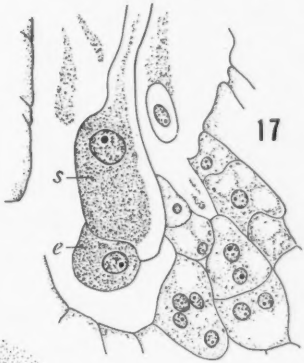

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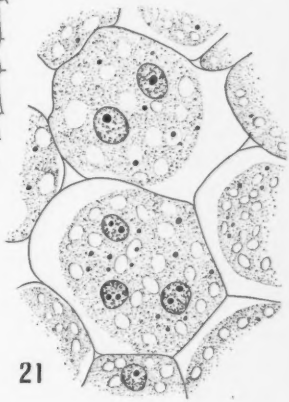

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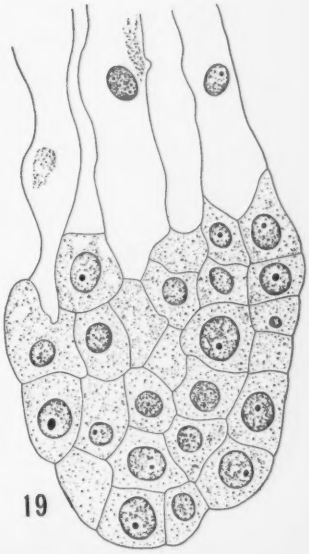

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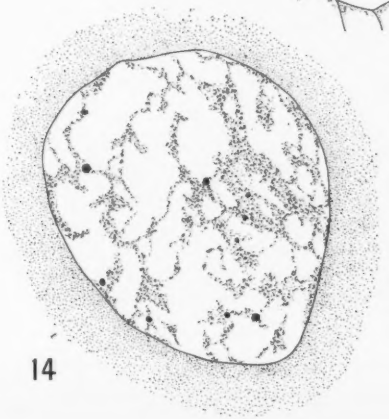

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12

THE BEHAVIOR OF THE CHROMOSOMES IN *OENOTHERA LATA*×*O. GIGAS*

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 128

REGINALD RUGGLES GATES

(WITH PLATES XII–XIV)

The hybrid which forms the subject of this paper is of peculiar interest because one of its parents has double the number of chromosomes possessed by the other, *O. lata* having usually 14 chromosomes and *O. gigas* 28. Very few cases of this sort are known, either in plants or animals. But a further complication arises in the fact that *O. gigas* is known to have originated from *O. Lamarckiana*, which also has 14 chromosomes, and *O. gigas* has in all probability attained the tetraploid number by a duplication of the chromosome set present in *O. Lamarckiana*.[1] Then if fertilization took place in the ordinary manner, the hybrid *O. lata*×*O. gigas* would be expected to have 21 chromosomes, 7 derived from the *lata* egg and 14 (which is probably a double set of *Lamarckiana* chromosomes) derived from the male cell of *gigas*. Under these circumstances, the behavior of the chromosomes in the hybrid, especially during the period of reduction and germ-cell formation, is a matter of especial interest.

The general results regarding chromosome numbers and distribution were obtained some time ago, and a brief statement was published (**10**), but the cytological evidence is here presented for the

[1] I have discussed this matter in another paper to appear in the *Archiv für Zellforschung*. DEVRIES in a footnote to a recent paper (**5**) considers it an important question whether the doubling in *O. gigas* is accomplished through a longitudinal or a transverse splitting. In all the cases, of which I am aware, of chromosome multiplication under experimental conditions without nuclear division (BOVERI **4**, LILLIE **23**), the chromosomes evidently divide longitudinally as in ordinary somatic mitoses so that it seems probable that the division in *O. gigas* has also been longitudinal (on the very probable assumption that it occurred after fertilization). This view is further supported by the fact that the chromosomes of *O. gigas* show no apparent change in size or shape from those of *O. Lamarckiana*. If the division had been transverse, such a change might perhaps be expected. It is not impossible that in *Drosera longifolia*, which has 40 small chromosomes (ROSENBERG **31**), while *D. rotundifolia* has 20 larger ones, there has been a transverse split of the *D. rotundifolia* chromosomes.

first time. Many of the drawings for these figures were completed about two years ago, but my interest in other phases of this work has postponed their publication until now. The earlier stages of reduction in Oenothera, up to the end of the heterotypic mitosis, have already been described in detail (**11**), so that this paper will deal chiefly with the later stages, beginning with the metaphase of the heterotypic mitosis.

The plants from which these studies were made were grown at Wood's Hole, Mass., in 1905 and 1906, from seeds of DEVRIES. The results show that in some cases the number of chromosomes is undoubtedly 21, while in one individual it was 20.[2] The number is undoubtedly constant in an individual, however, as shown by a large number of counts, which demonstrated constantly 20 in one case and 21 in the other.

The external characters were not studied with sufficient care at that time to describe them accurately, but from my notes they appear to have been intermediate between *O. lata* and *O. gigas*. DEVRIES has described in a recent paper (**5**) the hybrids of *O. gigas* with other forms. I have called attention elsewhere to the fact that the behavior of *O. gigas* in hybridization, as well as its number of chromosomes, places it in a different category from the other mutants of *O. Lamarckiana*. DEVRIES finds that *O. gigas*×*O. Lamarckiana* forms a constant race intermediate between the parents, at least to the second hybrid generation. *O. gigas*×*O. Lamarckiana*, *O. Lamarckiana* ×*O. gigas*, *O. gigas*×*O. brevistylis*, *O. gigas*×*O. rubrinervis*, and *O. rubrinervis*×*O. gigas*, all give constant hybrid races which are externally alike in all these crosses. *O. lata*×*O. gigas*, however, gives in the F_1 two types, about 50 per cent. of each; type I intermediate between *O. lata* and *O. gigas*, type II intermediate between *O. Lamarckiana* and *O. gigas*. These presumably would all have about 21 chromosomes. There were 133 plants in the culture of this hybrid in 1907 and a smaller number in 1908.

Miss LUTZ (**24**) from a study of 40 individuals of *O. lata*×*O. gigas* finds more complex conditions in this cross, though apparently she

[2] In my first paper (**8**) this individual was thought to be *O. lata*×*O. Lamarckiana*, but was afterward found to be derived from fertilization by foreign *gigas* pollen, this particular seed package not having been guarded as was supposed when the seeds were planted.

has failed clearly to recognize the types included in her class III. She divides the offspring into three classes. Class I has the characters of pure *O. lata*, and the two individuals which appeared are each said to have 15 chromosomes.[3] Class II consists of *O. gigas* plants having about 30 chromosomes. There were six of these plants, and their presence cannot be accounted for by the ordinary methods of fertilization. If the *O. gigas* male cell united with two *O. lata* nuclei in the embryo sac, this would account for the origin of the *O. gigas* number of chromosomes. But GEERTS (**12, 13**) finds that the embryo sac of Oenothera contains only four nuclei, the egg, two synergids, and one polar nucleus, so that the possibilities here are more limited than in an 8-nucleate sac.[4] The class III of Miss LUTZ apparently includes both the types of DEVRIES's cross, but they are not characterized so that a comparison can be made.

These interesting facts all show that there is still much to be explained regarding *O. gigas* and its hybrids. In the paper already referred to, I have shown that, in all the tissues examined, the cells are larger in *O. gigas* than in *O. Lamarckiana*, though the percentage of increase varies in different tissues. The production of the tetraploid number of chromosomes results in larger nuclei and larger cells, and this in turn in many cases produces larger organs. Certain changes which are found in the relative dimensions of the cells will account for the altered shape of certain organs. Thus the differences between *O. Lamarckiana* and *O. gigas* can probably be analyzed into two factors, (1) increased size of the cells, and (2) altered relative dimensions of the cells in certain cases. The former undoubtedly

[3] The presence of pure *O. lata* in this cross strongly suggests apogamy in this mutant, and it seems not unlikely that a condition exists resembling in part that found by ROSENBERG (**32**) in *Hieracium excellens*, in which part of the embryo sacs are developed after normal reduction processes and require fertilization, while occasionally embryo sacs are produced without reduction (apogamously or aposporously), in which case the egg develops without fertilization. I hope to test this hypothesis experimentally this summer.

[4] Whether an individual with the tetraploid number of chromosomes would have the *O. gigas* characters, owing to its *number* of chromosomes rather than their *source*, is a question I have considered in a forthcoming paper (footnote 1). The view that the number is the main factor is supported, perhaps, by the fact that all the mutants of *O. Lamarckiana* experimented with (except *O. lata* which is peculiar in its crosses), when crossed with *O. gigas* give a constant hybrid of the same type in every case.

results directly from the doubling of the chromosome number; at least the double number of chromosomes and the larger size of cells occur simultaneously. Whether the latter is a necessary consequence upon the increase in cell size and a resulting change in cell relations, or is due to an independent factor, is uncertain. It should be said that characters, such as leaf shape, which might be accounted for by a change in the relative dimensions of the cells (though I have not as yet made measurements of the leaf cells to determine this), are extremely variable, and it seems not unlikely that this extreme variability may result from variation in cell dimensions consequent upon the readjustment to the double chromosome number.

DESCRIPTION

The cytological account will begin with the telophase of the heterotypic mitosis in the pollen mother cell. The ten figures in plate XII are from the plant having 20 chromosomes, and were drawn on a smaller scale than those of the other two plates. At this stage the chromosomes can be counted with perfect accuracy. A large number of counts of this telophase show that 10 chromosomes enter each daughter nucleus. In a number of cases 10 were counted at each end of the same spindle. In several instances 11 were found in one of the daughter groups, and in a few cases it was possible to show that the corresponding daughter group contained only 9 chromosomes. In this plant, then, there are 20 chromosomes which segregate into two groups of 10 each in the reduction division, one chromosome occasionally going to the wrong group. Counts of somatic cells, made long ago in tissues of the anther, also showed that 20 chromosomes were present.

The material from which the figures in plate XII were drawn was subjected to an exceptionally high temperature in the process of imbedding, and in a few cases this has apparently affected the shape of the viscous chromosomes. *Figs. 1–5* are early telophases before the formation of a nuclear membrane. In *fig. 1* the chromosomes nearly all show their bivalent nature, and in most of them the two halves are dumb-bell shaped. This clubbing of the chromosomes at the ends is a common phenomenon both at this time and in the telophase of the homotypic and somatic mitoses. I have already

referred to these appearances in an earlier paper (**9**, p. 19). In *fig. 2* the chromosomes are more irregular in shape, and their bivalent character is not so evident. There are 11 chromosomes in the daughter group, and examination of the adjacent sections showed that there were 9 and no more at the other end of the spindle. The globular black bodies seen in the figure are frequently found scattered near the periphery of the cytoplasm. They stain like chromatin with Haidenhain's iron alum hematoxylin, but their chemical nature is unknown. *Figs. 3–5* each show 10 chromosomes. *Fig. 6* is a telophase with 11 chromosomes, the next section showing 9 at the other end of the spindle. *Fig. 1* is an exact polar view, the other figures being more or less oblique cuts of the spindle, but the spindle fibers are not represented. *Figs. 7, 8* are somewhat later stages in side view, soon after the nuclear membrane is formed. Ten chromosomes are present in each. In *figs. 9, 10* are shown in outline the loop-shaped chromosomes of two somatic cells. Each has 20 chromosomes. The cells are from the middle layers of the anther wall.

Plates XIII and XIV deal with plants having 21 chromosomes. *Fig. 11* is a side view of the heterotypic spindle, showing 20 or 21 chromosomes. It is usually difficult to count the chromosomes exactly at this time in a side view of the spindle, on account of the close aggregation of some of them, and the presence of spindle fibers. The chromosomes are almost never regularly oriented in an equatorial plate on the heterotypic spindle. An examination of the literature shows that in most plants and animals a flat equatorial plate is formed in the metaphase of this mitosis as in other mitoses, although a few forms constitute exceptions. But the homotypic mitosis in Oenothera has always a very definite equatorial plate, in which the chromosomes are oriented in a single plane. There can be little doubt that the irregularities in chromosome distribution arise from this failure of the chromosomes to be regularly paired and oriented on the heterotypic spindle. The irregularities in distribution certainly arise at this time.

I have recently confirmed practically all the events of reduction by a study of the wild *O. biennis*, so that this account of reduction applies to the genus Oenothera in general and is not the result of mutative conditions. This will be referred to again later.

Fig. 12 is another cell in telophase. One nucleus is uncut, showing 10 bivalent chromosomes. The other was sectioned by the knife. *Fig. 13* shows 11 chromosomes and a number of very small nucleoli. Usually only one or two larger nucleoli are present. In *fig. 14* there are 12 bivalent chromosomes. In such cases 9 appear at the opposite end of the spindle. *Figs. 17, 18* are very early telophases just after the nuclear membrane has been formed. The daughter nuclei grow very quickly to the size of *figs. 19, 20*, each of which shows 11 bivalent chromosomes, while in the nucleus represented in *fig. 21* there are only 9. In general I have found that the nucleus having 9 chromosomes is likely to be appreciably smaller than one having 11. Evidently the amount of karyolymph secreted by a nucleus is a function of its number of chromosomes. This is also shown by the many cases of single chromosomes left behind in the cytoplasm and forming small nuclei during reduction, as observed in various forms, especially in hybrids.

These drawings of telophases are nearly all from nuclei which are uncut by the knife in sectioning. The sections are usually 10 μ thick, so that in a majority of cases the nucleus is completely contained in one section. The nuclear membrane is conspicuous; hence by focusing it can be determined with certainty that the membrane is uncut and that the contents of the nucleus are intact. All the nuclei in which counts have been made have been first shown to be intact in this manner. Moreover, whenever the chromosomes in both daughter nuclei could be counted, their sum was invariably found to be 21, whether 10+11 or, as occurred in a few cases, 9+12.

There are a number of interesting features about the telophase, which I have studied with particular care. In the first place it is in the best stage for counting the chromosomes with absolute accuracy, and is even better than diakinesis, because of the reduced number of chromosomes. In my last paper on reduction (**11**) I showed that pairing before the reduction division only takes place to a limited extent, and that the shapes characteristic of heterotypic chromosomes are therefore usually absent at this time, as will be seen from *figs. 26–34* of the paper referred to. During the period of interkinesis, however, the entire chromosomes of the heterotypic mitosis having split to form bivalents in which the two parts are closely held together,

show the characteristic X, Y, V, H, K shapes, etc. (See *figs. 12–14, 19–21.*)

A large number of counts of the chromosomes were made in these telophases, and it was found that the numbers 10 and 11 occurred with approximately equal frequency, while the numbers 9 and 12 were only occasional. Every single case which was admitted as a count could be determined with absolute certainty as a case in which there were, for instance, just 10 chromosomes, no more and no less.

In the anaphase of the heterotypic mitosis, the chromosomes as they pass to the poles are nearly globular or somewhat elongated in shape, and are at first closely massed at the poles of the spindle. There is considerable variation in the time of appearance of the split in these chromosomes, though they usually appear bivalent in the early telophase. They appear to come into actual contact at this time, forming a compact group, but never fusing or uniting. They very soon begin to separate, however, and as they do so nuclear sap appears between and to a lesser extent around them. Then the nuclear membrane appears where the karyolymph comes in contact with the cytoplasm. The nucleus so formed is at first very small, but grows rapidly to its full size by the increase in nuclear sap.

LAWSON (**20**) has described this process in detail in *Passiflora coerulea*. He found that the chromosomes fused into a single mass in the telophase, and that the karyolymph begins to be secreted within this chromatic mass and later comes to surround it. The cytoplasm, coming in contact with the karyolymph, forms the nuclear membrane, which is therefore the limiting membrane of the cytoplasm, just as is the tonoplast of a vacuole. It is undoubtedly true that the nuclear membrane is formed where the karyolymph comes in contact with the cytoplasm, although it must remain uncertain whether the karyolymph is secreted by the chromosomes or merely attracted and accumulated about them from the cytoplasm. There must be extremely little if any cytoplasm included in the nucleus so formed, because the nuclear membrane appears close about the chromosome group when the chromosomes are still almost in contact. This supports GRÉGOIRE'S observations (**14, 15**), that the structural contents of the resting nucleus are formed wholly from the chromosomes, and there can be no doubt, in this case at least, that the

daughter nucleus is formed entirely from the chromosomes and the karyolymph in which they float. This is to me a strong observational evidence of chromosome continuity.

Unlike LAWSON's description for *Passiflora coerulea*, the chromosomes in Oenothera certainly do not fuse in the telophase of the heterotypic mitosis, but maintain their separate identity completely at first, and usually more or less completely thoughout the period of interkinesis. Occasionally stages are found which indicate that they string out and anastomose to some extent during a late stage of interkinesis, partly losing their sharp boundaries, but this stage apparently does not very often occur.

Nucleoli may be formed *de novo* in these daughter nuclei, as I have described previously (**8**, p. 93). The chromosomes in this telophase are all clearly bivalents, in which the halves are closely in contact. I have examined thousands of nuclei in this stage and have never seen the halves other than more or less closely in contact. On the other hand, it is universally true in my observations that no two chromosome bivalents are ever found in contact. Not only is this the case, but they are invariably distributed at approximately equal distances from each other, just within the nuclear membrane. I have never seen an exception to this in any Oenothera studied. The position of the chromosomes might be explained by supposing that they are attached to the nuclear membrane from the first, and are thus carried outward as the nucleus grows.[5] In many cases the chromosomes appear actually to be attached to the nuclear membrane, or at least to be lying very closely against it. This, however, leaves unexplained the fact that the chromosomes are never in contact with each other at this time, and further that they are very generally placed at approximately equal distances from each other, just within the nuclear wall. All these facts point to the supposition that the

[5] It might also be supposed that in the dehydration processes preparatory to mounting, the chromosomes would be drawn against the nuclear membrane. But in such a case one would undoubtedly find the chromosomes occasionally massed on one side of the nucleus or irregularly placed, instead of being always at regular intervals about the periphery. From the regularity of their placing I have no doubt that the chromosomes occupy their original positions within the nucleus, and there is no indication that they are ever disturbed by the processes of fixation, imbedding, and staining, when properly carried out.

chromosome bivalents are mutually repelled. It is true that in the early telophase the chromosomes form a close group, so that they certainly cannot be repelled at that time, but may be attracted. However, the medium in which bodies float frequently changes their qualities of attraction and repulsion, and it appears that the repulsion first develops after the appearance of the karyolymph in which the chromosomes float. The facts all suggest that the chromosome bivalents mutually repel each other at this time, while the halves of these are held together, probably by attraction.

The studies of WILSON (**37**) and others on insect chromosomes show that there are *selective* attractions between certain chromosomes at the time of synapsis or at some other period of meiosis. These of course require something more specific than electromagnetic forces to explain them.

Fig. 22 shows one of the nuclei of a pollen mother cell in the prophase of the homotypic mitosis. Eleven chromosomes are present, having the same bivalent structure as in the telophase of the previous mitosis. Both nuclei always go through the various stages of the second mitosis simultaneously. The method of spindle formation has not been studied with great care, but corresponds with the Gladiolus type of multipolar spindle formation (LAWSON **19, 21**). There is no indication of an intra-nuclear network, the spindle being wholly extra-nuclear in origin. A portion of the weft of fibers surrounding the nuclear membrane remains *in situ* when the latter disappears, forming a close meshwork, against which the chromosomes lie. The fibers then rearrange themselves; the fibrillae from the cones already formed come in and become attached to the chromosomes; finally the spindle becomes bipolar by the rearrangement of the fibers forming the cones.

The numerous papers on spindle formation in angiosperms need not be cited here. Among the more recent critical studies of this structure may be mentioned that of BERGHS (**2**), who insists that the distinction between kinoplasm and trophoplasm will not hold; that the spindle results simply from the gradual orientation of the material of the cytoplasmic reticulum, and returns to a reticulum after mitosis.

Sometimes spindle formation begins on one side of the nucleus, as in *fig. 23*. In such cases the cones of the multipolar spindle may

be formed and the nuclear membrane may have disappeared on one side of the nucleus before any indication of spindle formation has appeared on the other side.

Fig. 15 is a polar view of the two homotypic spindles in metaphase. There are 11 chromosomes on one spindle and 10 on the other. *Fig. 25* is another at the same stage, showing 10 chromosomes. In *fig. 24* the spindles are at right angles and all the chromosomes are not shown. In the side view of the spindle some of the chromosomes appear like tetrads, owing to their bivalent structure. On the homotypic spindle, before the chromosomes divide, they are very regularly oriented in a single plane in the equatorial plate, as in *figs. 15, 25*. This contrasts strikingly with the heterotypic spindle, in which the chromosomes are scattered for a considerable distance along the long axis of the spindle, so that there is usually no metaphase, strictly speaking.

Fig. 26 is an early anaphase of the homotypic mitosis, showing one spindle in side view and one in polar view. In the polar view 20 chromosomes are found by focusing through a short distance, and the remaining 2 are found on the next section. In the side view the chromosomes could not all be counted, but presumably there were 20 after division. Two pale-staining nucleoli still persist in the cytoplasm.

Fig. 16 shows three of the nuclei in the telophase of the homotypic mitosis. Many of the chromosomes have a characteristic two-lobed or dumb-bell shape. One chromosome is left behind in the cytoplasm, leaving 10 chromosomes each in two of the daughter nuclei. This two-lobed shape is a characteristic appearance of the chromosomes in the telophase of somatic mitoses, but presumably bears no relation to the next mitosis, because if this were the beginning of a split for the next mitosis it would indicate that the division of the chromosomes is transverse. But metaphase and anaphase stages of somatic mitoses show that the chromosomes divide longitudinally. Of course the possibility that the transverse axis of a chromosome in telophase should regularly become its longitudinal axis before the next metaphase, is not excluded, although this seems unlikely.

The great difference in the size of the figures made it impossible to arrange them in order on the plates. The magnification is the same

as in my last paper in this journal (**11**), so that the figures can be directly compared.

DISCUSSION

The history of any ontogeny is the history of the transformation of chemical metabolism into definite structures, or rather the eventuation of a series of chemical processes in the production of a series of physical structures. Morphologists and cytologists map the succession of structures appearing and call it a series of events. They are not unmindful, however, that the primary process is the metabolism, the structures its by-products, which in turn, so far as they are capable of continuing metabolism, produce other structures and, particularly in the adult organism, structures like themselves. Similarly, the chromosomes of the germ nuclei must be thought of as definite aggregations of chemical materials, which initiate or take part in certain forms of metabolism. The chromosomes themselves do not (at any rate not wholly) control their growth or division, but this is more or less subject to the conditions of temperature, etc., in which they are placed, as the work of ERDMANN (**7**) and others has shown. Similarly, as the complex of physical and chemical conditions in the nucleus and cell undergoes changes, the chromosomes change from the compact to the alveolate or distributed condition, or *vice versa*, etc. The cytologists who record these events are aware that chemical transformations are continually going on, and that the changes in visible physical structures are the external concomitant of such chemical processes.

Chemical reactions in the test tube, however complex, do not lead to the production of any structures more complex than crystals or flocculent masses of various sorts. There are indications that living matter frequently has the properties of liquid crystals, but why do the infinitely more complex reactions of living matter build structures of such relative permanency and of tremendous intricacy? The problem of individual development, from this standpoint, is the question, How do certain forms of chemical metabolism result in the production of certain visible physical structures or characters? Of course, our present microscopic appliances do not permit us to know how many structural steps, if any, there may be between the interacting molecular masses and the finest structures visible under our

highest powers, but it is by no means necessary to assume that there are such. This digression will indicate to some extent, perhaps, the viewpoint of the writer in connection with the cytological aspects of this work.

No attempt will be made to discuss here the literature of reduction, only a very few of the recent papers being referred to. The discussion of matters of cytological detail will be taken up at another time.

One important matter, which was discussed in a former paper (**11**), concerns the method of chromosome reduction, i. e., whether there is a pairing of threads about the time of synizesis and whether the spirem afterward breaks into a single series or two parallel series of chromosomes. In the paper referred to I established the fact (as will be conceded, I think, after a study of *figs. 20–32*, particularly, of that paper) that the spirem breaks into a single and not a double chain of chromosomes. The possibility of finding a series of stages which really demonstrates this depends on the shape of the chromosomes in Oenothera. They are relatively short and thick, like many animal chromosomes, and quite unlike the twisted and tangled chromosomes of such forms as Lilium, whose relationships are so difficult to interpret. It is a curious fact that so many of the critical studies on reduction in plants have been made on forms with long narrow chromosomes, although many of the interpretations of critical stages can be made with much greater ease and certainty on forms having short and stout chromosomes.

In the paper just referred to, I concluded that the method was probably different in different genera, there being a side-by-side pairing of threads in some forms (parasynapsis),[6] but an end-to-end arrangement of the chromosomes to form a single spirem (telosynapsis)[6] in other forms. Subsequent papers by various investigators continue to describe both these methods, and the evidence in certain cases is so clear that I think there can remain no doubt that both these general methods occur in plants. MONTGOMERY concluded in 1898 (**26**) that there are different types of reduction in animals.

In a recent study of Fucus, YAMANOUCHI (**39**) shows clearly that at the time of reduction the ragged nuclear reticulum is gradually transformed into a single continuous thread, which enters into

[6] These convenient terms follow the usage of WILSON (**36**).

synapsis. This thread then becomes rearranged into regular loops converging to one side of the nucleus and forming the "bouquet" stage of EISEN (**6**). This stage is characteristic of many animals, in which it has been described by JANSSENS and DUMEZ (**17**), STEVENS (**34, 35**), MARÉCHAL (**25**), and JORDAN (**18**), to mention only a few. Each loop is composed of two chromosomes arranged end to end. Similarly the spirem in Fucus is made up of the maternal and paternal chromosomes arranged endwise in a single thread. This is what I have shown to be the case in Oenothera (**11**), although Oenothera has no typical "bouquet" stage, nor has any other angiosperm, so far as I am aware, though the second contraction phase, characteristic of various forms, probably corresponds to it. OVERTON (**30**) states that this stage does not occur in the plants with short chromosomes which he has examined, yet it occurs in Oenothera, in which the definitive chromosomes are very short and frequently almost globular. The second contraction phase is a well-marked stage of meiosis in Oenothera.

I may mention a few of the recent accounts involving an end-to-end arrangement of the chromosomes to form the spirem (telosynapsis). MOTTIER finds (**29**) that in Podophyllum, Lilium, and Tradescantia the two members of each bivalent chromosome were not side-by-side in the spirem, representing the halves of the longitudinally split thread, but were arranged end-to-end in the chromatic thread. LEWIS (**22**), in a study of Pinus and Thuja, finds no pairing of threads, but cross-segmentation of a single post-synaptic spirem to form the chromosomes. Just what relation his *fig. 15*, which indicates a reticulum as occurring about the time of chromosome formation, bears to the other stages, it is hard to say. LEWIS ventures the opinion that the second division is probably qualitative, but with a manifest lack of evidence to support it. SCHAFFNER (**33**), in Agave, finds bodies which he believes are bivalent prochromosomes, and a single spirem which segments to form the twelve bivalent chromosomes.

Among very recent accounts of reduction which involve a longitudinal pairing of threads (parasynapsis) may be mentioned that of GRÉGOIRE (**16**), with figures involving the critical stages in Lilium, Osmunda, and Allium; YAMANOUCHI (**38**) in Nephrodium; OVER-

TON (30) in Thalictrum, Calycanthus, and Richardia. ALLEN'S extensive earlier paper on Lilium (1) should also be mentioned. Although, in such cases as Lilium, both accounts of reduction are given for the same form by different authors, yet the evidence from such forms as Nephrodium on the one hand, and Tradescantia, Oenothera, and Fucus on the other hand, makes it very difficult to deny that both methods occur. A comparison of a wide range of forms whose reduction phenomena show many differences, will doubtless lead finally to an explanation of the nature and meaning of these differences. It is very evident that the time has passed when all the accounts of reduction in plants can be brought under a single scheme. The task of the future will be to interpret the meaning of the differences observed in various forms. Are they matters merely of cell mechanics, or are they related to hereditary processes? It is not impossible that correlations will be found between the method of reduction in an organism and its type of hereditary behavior. In other words, the phenomena of reduction and the distribution of elements during meiosis may condition to some extent the hereditary behavior of a plant or animal. This is already known to be the case with sex in insects. But it does not seem worth while entering into such theoretical possibilities on the basis of our present knowledge.

The fact that in this Oenothera hybrid the number of chromosomes in the pollen mother cells is the sum of those which enter the fusion nucleus at the time of fertilization, is to me clear evidence that each chromosome in the germ cells is the genetic descendant of one of the chromosomes in the fertilized egg. The work of various investigators seems to have established the genetic continuity of chromosomes from generation to generation of individuals. The manner in which the chromosomes are distributed during reduction in this Oenothera hybrid clearly shows that they behave as individuals at this time. WILSON'S recent account of the supernumerary chromosomes in Metapodius (37), a genus of Hemiptera, shows certain points of resemblance to the condition in Oenothera. WILSON finds that certain chromosomes (the idiochromosomes) may be present in duplicate, or may even have several representatives in the cells of certain individuals, which nevertheless show no external differences, the number of chromosomes being fixed for any individual, but varying

in different individuals from 21 to 28 according to the number of supernumerary chromosomes present. The distribution of the supernumeraries during spermatogenesis is also irregular, so that the number varies in the different sperm nuclei of an individual. Occasional irregularities in the distribution of idiochromosomes during reduction were observed in the form having 22 chromosomes, both idiochromosomes passing to the same cell. This is believed to be the origin of the variation in different individuals, the supernumeraries being merely duplicates of the idiochromosomes.

Similarly, I have observed occasional irregularities in the chromosome distribution during reduction in nearly all the mutants of Oenothera examined, and this doubtless accounts for the different numbers of chromosomes found in different individuals, the extra chromosomes being duplicates of others already present, and the presence of 20 chromosomes in one individual of *O. lata*×*O. gigas* being due to the absence of one chromosome from one of the germ cells which produced that individual.

MONTGOMERY (**27**) first proposed the theory that in synapsis homologous chromosomes of maternal and paternal origin pair with each other, and much subsequent work, especially with animal chromosomes, sustains that view. As I have already shown, pairing of Oenothera chromosomes frequently fails to take place, and this allows a chance for the irregularities in chromosome distribution which occur. BOVERI (**3**, p. 54) has suggested that when there is an absence of pairing in synapsis in any organism, its chromosomes are hereditarily equivalent. In Oenothera there is clearly a tendency to pair, but it is only partly carried out. I have already compared (**11**, p. 25) the behavior of the chromosomes in this hybrid with the condition described by ROSENBERG (**31**) in the Drosera hybrid having 10+20 chromosomes. The method of segregation of chromosomes in the heterotypic mitosis is evidently different in the two hybrids, and this was used as an argument in favor of a different method of reduction in the two cases. In the Oenothera hybrid the 10–11 segregation shows that the segregation cannot be between chromosomes of maternal and paternal origin, but it must be remembered in this connection that the 14 paternal chromosomes are very probably a double set of 7 *O. Lamarckiana* chromosomes. The regu-

larity with which the 10–11 segregation takes place, indicates that it is not merely a matter of chance, but that some mechanism, perhaps connected with the spindle, determines this regularity. A study of later generations of this hybrid should throw much light on the question whether the chromosomes of Oenothera are really unlike.

MORGAN (**28**) has recently shown that in certain phylloxerans, in which a generation of winged individuals produces (parthenogenetically) sexual males and females, the eggs are of two kinds, the female-producing eggs being large and the male-producing eggs small. Further, the females thus produced have the same number of chromosomes as the parthenogenetic females, while the males have two chromosomes less. Thus, in the formation of the polar body of the male egg, two extra chromosomes are extruded, so that the somatic cells of the male contain two less chromosomes than those of the female. Evidently there is here some sex-determining factor which antedates the chromosomal differences with the two kinds of eggs, and yet the chromosomes are the instruments of this factor, for the extrusion of the two chromosomes always precedes the development of the male individual. Studies of this sort will doubtless give us clearer notions regarding the respective rôles of chromosomes and cytoplasm in heredity.

In a former paper (**11**) I suggested that if the chromosomes of Oenothera are unlike in their hereditary capacities, then the occasional irregularities I have described in the chromosome distributions on the heterotypic spindle would furnish a possible basis for the appearance of a series of types, such as the mutants of *O. Lamarckiana*. I have since studied the reduction phenomena in *O. biennis* and other types, an account of which will be published later. *O. biennis*, in at least some of its geographical races, appears to be stable under ordinary conditions, though MACDOUGAL (**24a**) has obtained atypical forms by injections into its ovaries. The loose and frequently unpaired arrangement of the chromosomes in the central region of the heterotypic spindle is evidently a delicately adjusted condition which can easily be thrown out of balance. Yet in some way the balance is ordinarily maintained and is only occasionally deranged in such a way that a different chromosome distribution results. It is not impossible that the condition of mutation in *O. Lamarckiana*

has arisen through a disturbance by some means of this delicate condition of balance.

But it is useless to speculate on such possibilities until it is known whether the chromosomes of Oenothera are really unlike, and at present there is no evidence in favor of this view except the inferential evidence from chromosomes in general. The fact that chromosomes reappear with each mitosis, showing the same differences where visible differences exist (except in the cases of amitosis, whose status in relation to hereditary processes is not at present understood), would seem to favor the assumption that they maintain their identity and are unlike. But I need not cite here the general arguments in favor of this hypothesis. The more definite evidence, such as that of the sex chromosomes in insects, is not necessarily of universal application.

SUMMARY

1. *O. lata*×*O. gigas* has 21 chromosomes in its somatic cells, 7 of maternal origin (*O. lata*) and 14 of paternal origin (*O. gigas*). In one individual the number was 20, owing probably to the absence of one chromosome from one of the germ cells which produced this individual.

2. These chromosomes segregate at the time of reduction, so that in individuals having 21 chromosomes half the germ cells receive 10 and half 11 chromosomes. In the individual having 20 chromosomes, 10 enter each germ cell. Occasionally one chromosome goes to the wrong pole of the spindle, so that in plants having 21 chromosomes a few germ cells are found having 9 or 12 chromosomes and in the plant with 20 chromosomes, occasional germ cells have 9 or 11 chromosomes. This irregularity in chromosome distribution accounts for the fact that different individuals in a race in some cases have different numbers of chromosomes.

3. The 10–11 segregation of chromosomes in the formation of the germ cells of this hybrid shows that there is not here a pairing and separation of homologous chromosomes of maternal and paternal origin, but that the segregation tends to be into two numerically equal groups.

4. Evidence from this and other work shows that there are two general methods of chromosome reduction in plants, one involving

a side-by-side pairing of chromatin threads (parasynapsis) to form a double spirem; the other involving an end-to-end arrangement (telosynapsis) of the maternal and paternal chromosomes to form a single spirem, which may afterward split longitudinally.

5. The behavior of the chromosomes in Oenothera supports the view of their genetic continuity from generation to generation, the number present in any individual being always the sum of the chromosomes in the germ cells from which that individual was formed.

6. If the chromosomes of Oenothera are unlike in their hereditary capacities their behavior furnishes a not improbable basis for the phenomena of mutation in *Oenothera Lamarckiana*. But it remains to be proven that the chromosomes of Oenothera are of unequal hereditary value.

THE UNIVERSITY OF CHICAGO

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EXPLANATION OF PLATES XII–XIV

The figures were drawn with the aid of a Bausch & Lomb camera and a Zeiss apochromatic objective 2^{mm}, ap. 1.30, particular care being taken to represent the chromosomes accurately. The figures in plate XII were drawn with a Zeiss compensating ocular 12, those of plates XIII and XIV with ocular 18. All are reduced one-fourth in reproduction, which leaves the figures in plates XIII and XIV magnified as in my last paper (**11**) on reduction.

PLATE XII

All figures from the plant having 20 chromosomes.

FIG. 1.—Early telophase of the heterotypic mitosis in the pollen mother cells before formation of the nuclear membrane; 10 chromosomes, all clearly bivalent; in many the halves are dumb-bell shape.

FIG. 2.—Same stage; 11 chromosomes, many irregular; 9 chromosomes at the opposite end of this spindle; dark staining bodies in periphery of cytoplasm.

FIG. 3.—Telophase, showing 10 chromosomes of various shapes.

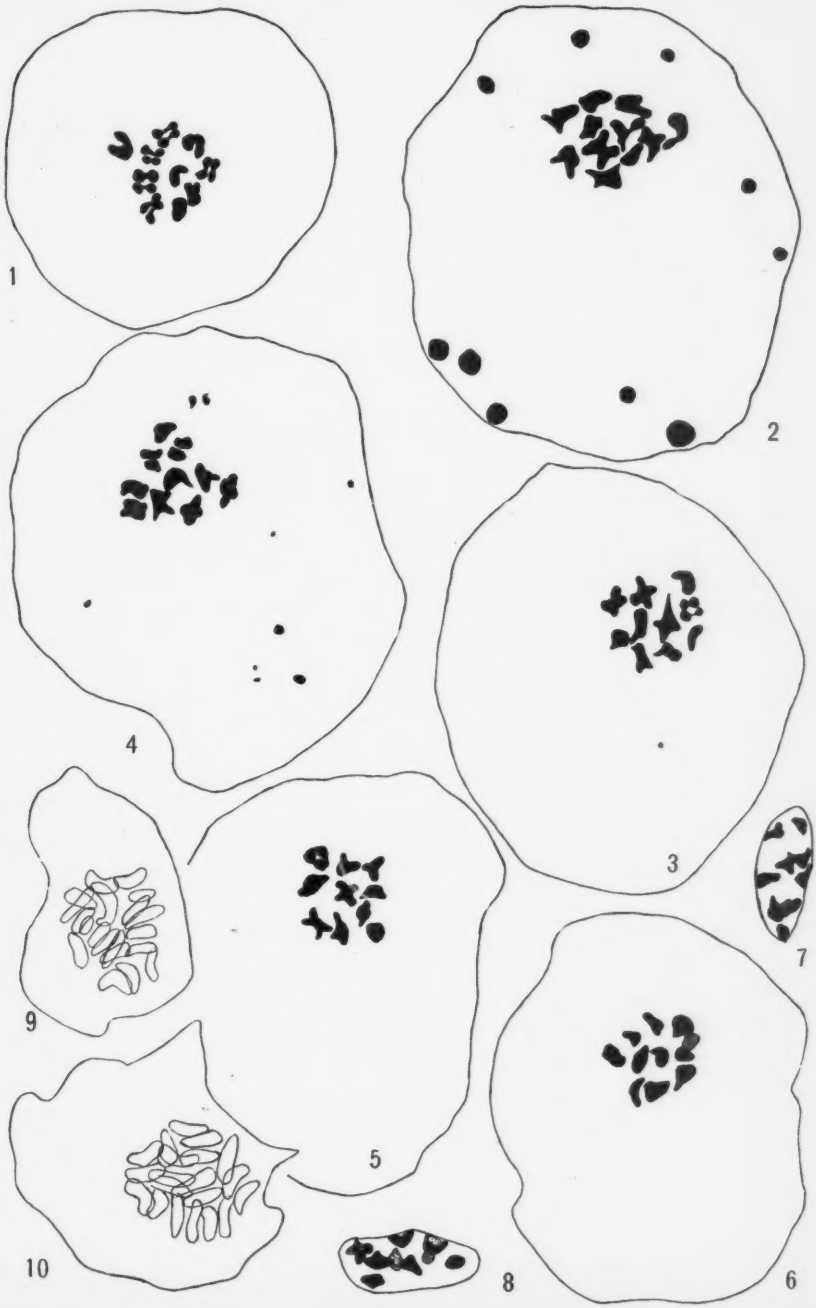
FIG. 4.—Telophase, showing 10 chromosomes, most of them clearly bivalents.

FIG. 5.—Same as *fig. 4*.

FIG. 6.—Telophase, showing 11 chromosomes, with no indication of their bivalent character; 9 at opposite end of spindle; apparent size of chromosomes in all figures varies to a certain extent according to the depth of stain.

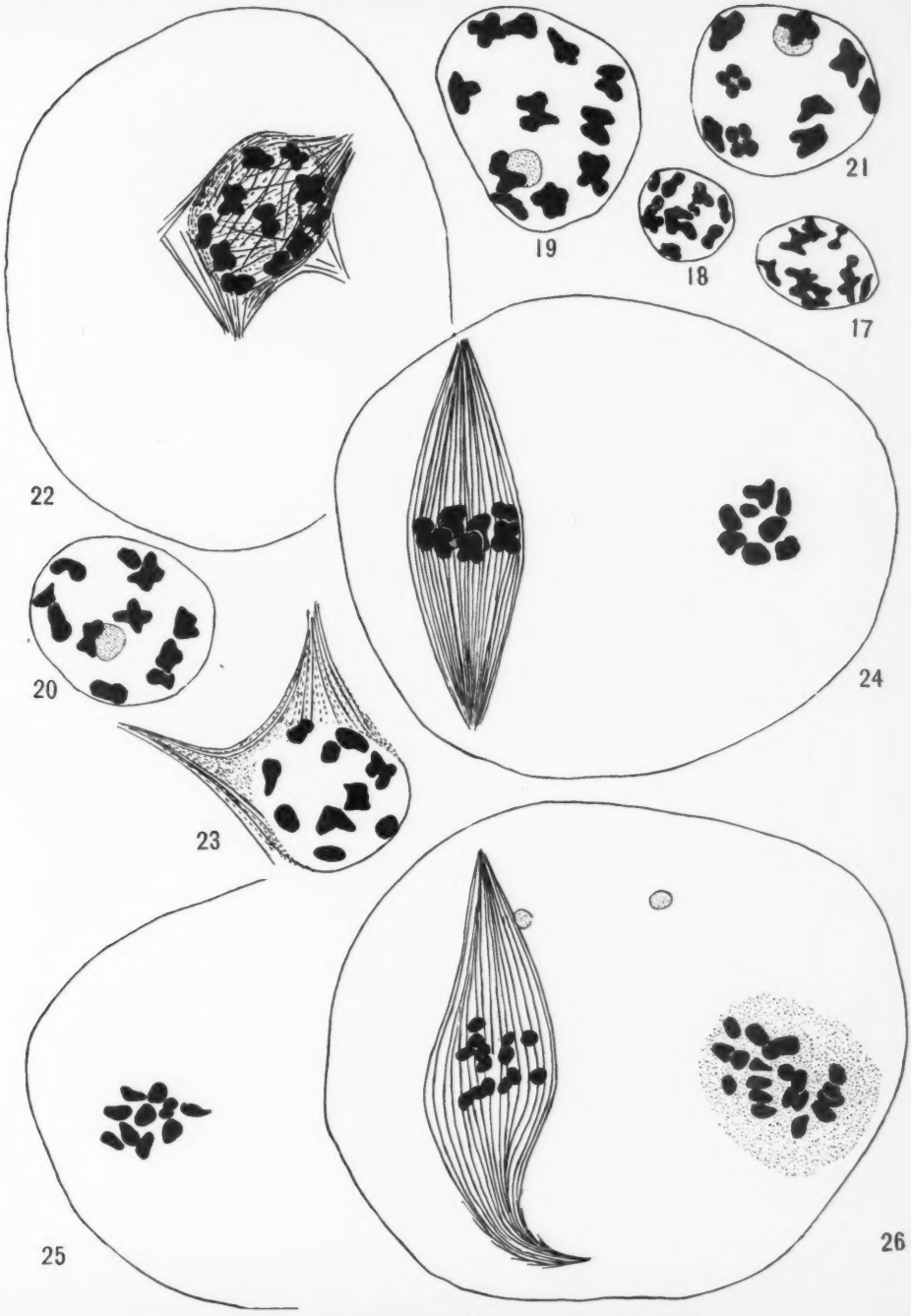
FIGS. 7, 8.—Later telophases, soon after formation of nuclear membrane, each showing 10 chromosomes.

FIGS. 9, 10.—Metaphase groups of chromosomes in somatic cells from middle layers of anther wall, each showing 20 chromosomes; indications of an arrangement of the chromosomes in pairs.



GATES on CHROMOSOMES of OENOTHERA

GATES on CHROMOSOMES of OENOTHERA



GATES on CHROMOSOMES of OENOTHERA

PLATE XIII

Figures in plates XIII and XIV from plants having 21 chromosomes; not numbered in developmental order.

FIG. 11.—Heterotypic spindle in side view, showing 20 or 21 chromosomes, not forming an equatorial plate, but loosely aggregated in the median region of the spindle; cf. *fig. 15*.

FIG. 12.—Telophase of heteroptypic mitosis, showing both daughter nuclei; one, uncut, shows 10 chromosomes.

FIG. 13.—Telophase, showing 11 chromosomes and a number of very small nucleoli; an unusual condition, probably due to the failure of the nucleoli to fuse at an earlier stage.

FIG. 14.—Telophase, showing 12 bivalent chromosomes, lying closely against the wall, tipped at various angles to the plane of view, which gives them a variety of appearances; an exceptional case, in which one chromosome too many has passed to the end of the spindle; 9 at opposite end; 2 nucleoli.

FIG. 15.—Metaphase of homotypic mitosis in polar view, showing equatorial plates of chromosomes, 10 chromosomes on one spindle and 11 on the other; cf. *fig. 11*.

PLATE XIV

FIG. 16.—Telophase of homotypic mitosis, showing three daughter nuclei; one chromosome left behind in the cytoplasm, leaving 10 chromosomes each in two of the daughter nuclei; many chromosomes show characteristic dumb-bell shape.

FIGS. 17, 18.—Very early telophase of the heterotypic mitosis, just after the nuclear membrane has been formed around the daughter nuclei; one shows 10 bivalent chromosomes, the other 11.

FIGS. 19, 20.—Telophases, each showing 11 bivalent chromosomes.

FIG. 21.—Telophase with 9 chromosomes only.

FIG. 22.—Prophase of the homotypic mitosis, showing 11 bivalent chromosomes, which have the same appearance as in the previous telophase; nuclear membrane just broken down, its position still occupied by a weft of fibrillae, against which the chromosomes lie; cones of the multipolar spindle forming.

FIG. 23.—Same stage as *fig. 22;* a less common condition, in which spindle formation begins on one side of the nucleus; 10 chromosomes, only a few of which show bivalent character.

FIG. 24.—Metaphase of the homotypic mitosis, showing one spindle in side view and the other in polar view; chromosomes not all shown.

FIG. 25.—Equatorial plate of homotypic spindle, showing 10 chromosomes in a single plane, few showing their bivalent character.

FIG. 26.—Early anaphase of homotypic mitosis, after division of the chromosomes; one spindle in side view, showing only part of the chromosomes (probably 20 in all); the other spindle in polar view, showing 20 chromosomes (two more are on this spindle in the next section); 2 nucleoli in the cytoplasm near the spindles.

THE DEVELOPMENT OF THE EMBRYO SAC OF *SMILACINA STELLATA*

F. McALLISTER

(WITH PLATE XV)

The object of this paper is to describe the development of the embryo sac of *Smilacina stellata* (L.) Desf., with a view to its possible bearing on the current interpretations of the lily type of embryo sac.

In 1880 TREUB and MELLINK (**27**) reported that in *Lilium bulbiferum* and in *Tulipa Gesneriana* the embryo sac mother cell develops directly into the embryo sac without any previous divisions.

In 1884 GUIGNARD (**11**) and also STRASBURGER (**24**) called attention to the fact of a reduction of the number of the chromosomes during the development of the germ cells of angiosperms. STRASBURGER (**25**) further pointed out in 1888 that in the case of the embryo sac in certain orchids and in Allium, the reduction of the number of the chromosomes occurs in the nucleus of the embryo sac mother cell.

It was further established simultaneously by OVERTON (**16**) and GUIGNARD (**12**) that, in the lilies and other plants in which the embryo sac mother cell develops directly into the embryo sac without previous division, reduction takes place in the nucleus of the young embryo sac. The natural conclusion from these discoveries is that the young embryo sac in these cases is the morphological equivalent of a pollen or embryo sac mother cell. That the nuclei resulting from the first two divisions in the embryo sac of the lilies are morphological equivalents of the microspores is strongly suggested by these results.

STRASBURGER (**26**) in 1894, discussing the formation of the embryo sac of the lilies, concludes that the cell in which the reduction of the chromosome number takes place is to be regarded as a mother cell and not simply as a young embryo sac, since in the ovaries of Allium and Helleborus he had found that the reduction of the chromosomes takes place in the embryo sac mother cell before it has

undergone division. He concludes that in these cases the course of development is abbreviated, so that there is no formation of reduced cells which are to be immediately absorbed, as is the case in many other species. According to this view the heterotypic and homeotypic divisions are transferred to the early stages of development of the gametophyte.

The term "macrospore" or "megaspore" is frequently loosely used by the supporters of this view in reference to the cell which develops into the embryo sac, whether it be the young embryo sac itself, or one of the daughter cells, or one of four granddaughter cells which have been formed by the reduction divisions.

From the evident homology of the nuclei of the first two divisions of the embryo sac mother cell of the lilies with the microspores, as suggested by the discoveries of GUIGNARD, STRASBURGER, OVERTON, and also later investigators, the obvious interpretation of their nature is that they are megaspores. According to this view the reduction divisions may be regarded as the sole criteria of spore formation.

STRASBURGER (**26**) further says, in reference to the significance of the number of divisions which intervene between the embryo sac mother cell and the completed embryo sac, with its egg: "That the number of these intervening divisions is not of primary importance is proved by the fact that the number is not always the same: thus in Lilium and Tulipa there are but three; in Ornithogalum, Commelyna, and species of Agraphis, there are four; in yet other cases the number is greater than five. . . . ."

Smilacina stellata, the species which I have studied, is a member of the order Convallariaceae. The data as to the morphological relationships of the genus Smilacina are scanty and in part contradictory. The order Convallariaceae is retained by ENGLER and PRANTL under the group Asparagoideae, but the nature of the relationship of the group to the other orders of the Liliales is not very clear. The other members of the Convallariaceae whose embryo sac development has been studied are Convallaria, Paris, and Trillium.

Convallaria majalis has been investigated, as to the development of its embryo sac, by WIEGAND (**29**). He reports that the embryo sac mother cell divides to form two fully separated daughter cells, the outer of which is the larger. The nuclei of both of these cells

undergo a second division, but this time no cell walls are formed. The resulting four nuclei again divide, and the partition wall between the two sets of nuclei disintegrates enough to allow a nucleus from the lower set to pass through and unite with one from the upper set. This fusion nucleus is the endosperm nucleus. If this account is correct, we have at least a partial absorption of a cell wall in the formation of the embryo sac and the utilization of all four nuclei of the double division, as will be described below for *Smilacina stellata*.

WIEGAND'S account is contradicted, however, by SCHNIEWIND-THIES (**21**), who reports that in *Convallaria majalis* the mother cell divides to form a row of four cells, one of which develops into the embryo sac, while the other three disintegrate. As a possible explanation of the difference which exists between her account and WIEGAND'S, she remarks that greenhouse material rarely shows normal development. WIEGAND, however, does not mention the use of such material in his investigation. That both WIEGAND and SCHNIEWIND-THIES are correct is possible, but not very probable. It is very much to be desired that this species be reinvestigated to clear up this confusion.

ERNST (**9**) reported that in *Paris quadrifolia* the lower of two daughter cells develops into the embryo sac. The upper daughter nucleus divides once, but the resulting nuclei degenerate. In the same paper he reports that the lower of two daughter cells of *Trillium grandiflorum* develops into the embryo sac. The upper daughter cell is smaller from the first and rarely divides. For *T. recurvatum*, on the other hand, CHAMBERLAIN (**6**) has reported that the embryo sac develops from the lower of four megaspores.

Though scanty, the literature on the order Convallariaceae suffices to suggest that there exists in the group great variation in the conduct of the cells and the nuclei resulting from the reduction divisions, and that we may here find transition conditions between the so-called normal type of embryo sac formation and that found in the lily.

The materials for this study were collected in the vicinity of Beloit, Wis., in May 1906 and 1907, in November 1907, and in June 1908. Flemming's strong solution gave the best fixation for the early stages, but worked badly for mature embryo sacs. With these mature embryo sacs the following chromacetic fixative gave good results:

chromic acid 0.7^{g}, glacial acetic acid 0.5^{cc}, water 100^{cc}. A fixative composed of two parts absolute alcohol and one part glacial acetic acid gave very good results also. Flemming's weak solution was not tried.

Smilacina stellata is especially favorable for obtaining a complete series of stages of the early development of the embryo sac. The flowers, eight to fourteen in number, are borne in a raceme, which does not expand till the embryo sac has nearly reached the eight-celled stage. It is therefore possible to cut an entire raceme longitudinally and get a maximum number of ovules cut parallel to their long axes. The oldest flowers are at the bottom. Each flower contains five to seven ovules, and about seventy racemes were cut in paraffin.

In the material collected November 16, the nucellus was only partly developed in the lower flowers of the raceme. There were no mother cells to be distinguished in any of this material. Material collected May 7 showed, in a few shoots, mother cells in the synapsis stage in the lower flowers, while at the top of the shoot the nucellus was barely differentiated. Most of the racemes taken at this date were farther developed than those mentioned above, the younger flowers being at least in the synapsis stage.

The mother cell is located at a variable depth beneath the surface of the nucellus. Not uncommonly it is immediately beneath the epidermis (*fig. 1*). In other cases it is separated from the epidermis by one cell layer. Most commonly two cell layers intervene (*fig. 2*). Measurements of several camera drawings of the mother cell at the synapsis stage gave an average breadth of 22 μ, and an average length of 30 μ.

I shall not here take up in detail the question of the reduction of the chromosomes. In the first division of the mother cell, thick double chromosomes, characteristic of the metaphases of the heterotypic division, appear (*fig. 3*). The number of bivalent chromosomes, as shown in the anaphase stage of this division, is twelve. The sporophytic number, as shown by root tip cells, is twenty-four.

A definite cell plate, and in most cases a cell wall, is formed, separating the two daughter nuclei of the first division before the second division takes place (*figs. 4, 5*). The cell plate is formed by

the thickening of the connecting fibers in the equatorial region to form a septum which separates the cells. This splits in the central region first, and often a thin layer of orange staining material is to be seen between the split layers. The plasma membranes formed by the splitting of the cell plate are made very conspicuous in cells in which there is a slight plasmolysis. The cell wall seems in most cases to be fully formed before the second division is completed. Several preparations were found in which the wall seemed to be incomplete after the second division (*fig. 7*). These, however, may represent a stage in the removal of the cell walls, as described below.

In many cases the wall of the first division is transverse, but not infrequently it is oblique (*figs. 4, 12*). The walls formed in the second division are extremely variable as to their position. Frequently (*fig. 11*) they are transverse, forming a linear row of four cells. Very often the outer daughter cell divides longitudinally and the inner transversely (*fig. 8*). Less frequently, the outer divides transversely and the inner longitudinally (*fig. 6*). *Fig. 7* shows an arrangement which is occasionally met with, both daughter cells having divided longitudinally. Rather frequently one section shows two cells divided transversely, while the next succeeding section shows two cells divided longitudinally (*figs. 9, 10*). This arrangement could only result from the division of the mother cell at first by a longitudinal wall parallel to the plane of the section, and the division of one of the daughter cells by a transverse wall and of the other by a longitudinal wall. This case is easy to recognize when the plane of the section is such that both of the cell boundaries of the second division are vertical. It is much more difficult to recognize when one of the cell boundaries of the second division lies in or near the plane of the section. Such a mode of division is of course frequently found in pollen mother cells. *Fig. 12* shows, however, by far the most common arrangement of the cells, in which the first wall is oblique and the walls of the dividing daughter nuclei are approximately at right angles to it.

Most of these various arrangements of the four cells resulting from the division of the embryo sac mother cell have been described by other authors, but in the stages immediately following we are

confronted with a series of changes leading to a method of embryo sac formation quite different from any which has been hitherto described.

The cell walls which separate the four megaspores break down and disappear. Immediately following the formation of four fully separated daughter cells and nuclei (*figs. 8, 11*) we find a stage in which the same four nuclei are seen occupying a large cell with no traces of cell walls separating them (*fig. 14*). The evidence of the disappearance of the walls between the megaspores is not based on scattered examples, selected from a large number of specimens, but on a large number of continuous series of stages taken from a single inflorescence.

Fig. 11 shows four cells fully separated by cell walls, while in the next older flower on the same shoot are found four nuclei in a common cavity (*fig. 14*). In *fig. 13* the cell walls formed by the second or homeotypic division have entirely disappeared, while a slight but distinct cleft, extending across the cell, shows the location of the wall formed between the nuclei in the first division.

A statistical examination of a number of racemes to determine the exact stage of development of the mother cell or its products in each ovule, gave very conclusive evidence of the continuity of these series. The following is a fair example of an average shoot of the proper age. In this shoot of nine flowers the youngest flower had mother cells in a stage later than synapsis. The next older flower contained mother cells in the prophase and metaphase stages of the first division. The third showed in one ovule a heterotypic anaphase stage, and the other ovules showed the heterotypic telophase stages. The fourth flower showed the earlier stages of the second or homeotypic division. The fifth contained daughter nuclei of the second division in the telophase stage, and the sixth showed nuclei of this division separated by cell walls. The next older flower showed in a part of its ovules the four nuclei still separated by cell walls, while the rest of its ovules showed little or no trace of cell walls between the four nuclei. The ninth flower, the oldest on the raceme, showed four-celled embryo sacs in all its ovules, with no traces of cell wall separating the nuclei. The series here described shows no trace of the growth of one megaspore at the expense of the other three, or of the

first and second divisions of such a megaspore to form a four-celled embryo sac. We must conclude, therefore, that the walls between the four megaspores break down, and their nuclei become the first four nuclei of the embryo sac.

In the succeeding stages there is a continuous growth of the nuclei resulting from the reduction divisions and of the embryo sac formed from these nuclei. Very soon after the cell walls have disappeared from between the reduction nuclei, vacuoles begin to appear in the young embryo sac. *Figs. 15, 16, 17* illustrate the gradual enlargement of the cell containing the four nuclei, and the appearance of vacuoles in the cytoplasm. The vacuolization more than keeps pace with the enlargement of the embryo sac, and finally the separate vacuoles unite to form one large centrally located vacuole. It is at about this stage of growth that the third division takes place, forming the eight nuclei of the complete embryo sac (*fig. 17*). The embryo sac still continues to enlarge. The nuclei remain unchanged for a relatively long period. The polar nuclei come together and lie in contact during this period of quiescence, but are seen to be fused before the cells in the micropylar region show any signs of differentiation to form the egg apparatus. Not until nearly time for pollination is there any rearrangement of these cells, which are to form the egg apparatus.

When finally differentiated the synergids are pear-shaped and faintly striated. The egg is somewhat larger than the synergids, and usually has a large vacuole in the basal region. It stains less heavily than the synergids. I shall not here discuss the formation of the cell boundaries of the synergids and the egg. The antipodal nuclei are smaller than the others, and usually occupy a constricted region at the lower end of the embryo sac. These antipodal nuclei at this stage often number more than three, and in such cases are usually separated by division walls.

The most natural interpretation of the phenomena just described is that the first four cells formed by the division of the embryo sac mother cell are megaspores, and that these four spores jointly combine to form one embryo sac. It would seem that this assumption is the only one possible, for before the division membranes disappear, the four cells conform to all the criteria for spores in other similar

cases, and the mere loss of the division membranes cannot affect their morphological value.

Some preliminary studies of *Smilacina racemosa* seem to show that the two outer nuclei, formed in the double division of the embryo sac mother cell, undergo two further divisions to form the eight nuclei of the mature embryo sac. I shall describe this species, with other related species, more fully in a later paper.

Recent studies have brought to light an increasing number of so-called atypical methods of embryo sac formation, which must be considered in attempting its phylogenetic interpretation.

In Eichhornia, according to SMITH (**22**), a cell plate is rarely formed between the two nuclei resulting from the first division of the embryo sac mother cell, and a cell plate is also rarely formed between the daughter nuclei of the second division. Reduction takes place and one of the four megaspores forms the embryo sac.

CAMPBELL (**2, 3**) reported the discovery of the 16-nucleate embryo sac of *Peperomia pellucida*, and in the following year JOHNSON (**13**), working independently, published an account of the same species. The embryo sac mother cell develops directly into the embryo sac. The sixteen nuclei organize to form an embryo sac with an egg apparatus consisting of an egg and one synergid. Six nuclei are cut off singly around the periphery of the embryo sac, and the remaining eight nuclei fuse to form the endosperm nucleus. Later JOHNSON (**14**) reported that in *Peperomia hispidula* fourteen out of the sixteen nuclei of the embryo sac unite to form the endosperm nucleus.

In contrast with the large number of nuclei in the embryo sac of Peperomia, is the embryo sac of *Helosis guayanensis*, which is reported by CHODAT and BERNARD (**7**) to contain only four nuclei when mature. This 4-nucleate embryo sac is due to the disintegration of the lower nucleus of the first embryo sac division, so the products of the division of the upper nucleus alone enter into the embryo sac structure.

Avena fatua has been shown by CANNON (**5**) to form its megaspores similarly to Eichhornia. Commonly no cell walls are formed between the four spore nuclei, but the lower of these nuclei develops into the embryo sac and the other three degenerate.

A case resembling that of Avena and Eichhornia is reported as

occurring in Crucianella by LLOYD (**15**). He reports that in three species of Crucianella investigated, four megaspores were formed which were not separated by cell walls, and that "only occasional exceptions could be found to this." The *upper* of these four nuclei develops into the embryo sac, and the three lower finally degenerate. Not uncommonly these three lower nuclei undergo division. Many cases were seen, LLOYD reports, in which the four megaspores had each divided once, thus forming eight nuclei in a common cavity. He says: "If these divisions are regarded as the first mitoses of an embryo sac we have four embryo sacs lying tandem." Only the one lying adjacent to the micropyle attains full development. Although he reports the disintegration of the lower megaspores, his figures admit other interpretations. As late as the third division of the functional megaspore, what he interprets to be one of the three lower megaspore nuclei is shown dividing in the same cavity with the four dividing nuclei of the embryo sac. The dividing nuclei seem to have the same appearance in every way. This would certainly show a prolonged activity on the part of these three inner megaspores. This delayed germination of the lower megaspores and the similarity of the dividing nuclei in every way, open up many possibilities. The exact fate of the eight free nuclei formed by the division of all four of the megaspores is left unsettled. LLOYD concludes that the outer megaspore attains full development, and that the eight nuclei therefore never organize to form an embryo sac jointly. This is a most interesting case, and a complete history of the embryo sac from the mother cell may throw light on the interpretation of multinucleate embryo sacs.

This omission of the division walls between the megaspores of a tetrad is found by LLOYD also in Asperula, which is related to Crucianella. Any one of four spores may germinate to form the embryo sac, and the other three finally degenerate. The three spores which do not germinate are at times difficult to distinguish from antipodal nuclei.

An important conclusion to be derived from the behavior of Eichhornia, Avena, Crucianella, and Asperula is that the division walls are not essential to the individualization of a spore, and that the failure to form division walls between the megaspores does not

necessarily result in the lily type of embryo sac, even though the nuclei seem to be of the same size and vitality. So long as the spore retains its individuality as such, we can expect each spore to develop into a separate gametophyte, and not till this individuality is lost can we expect to find more than one spore entering into the structure of a single gametophyte. This absence of division walls, however, may very well lead directly to such joint organization of a gametophyte as we find in Smilacina.

LLOYD, in discussing the nature of the first four nuclei of the embryo sac of the lily, is in favor of calling them spores. "But after all," he says, "spores in the sense meant here are equivalent to vegetative cells of a somewhat special sort, with no necessarily separate existence teleologically speaking." He urges against the idea that in the lilies the gametophyte begins with the mother cell this: "It would seem more natural to regard the gametophyte as an individual by coalescence, having its origin in four like vegetative cells whose primitive function has been lost."

In *Pandanus Artocarpus* and *P. odoratissimus*, according to CAMPBELL (**4**), the mother cell develops directly into the 14-nucleate embryo sac. The two outer nuclei formed by the reduction divisions do not divide, while the two inner nuclei divide to form twelve nuclei. A differentiation of the antipodal cells was reported, but no certain evidence of nuclear fusions to form the endosperm nucleus was found.

In 1907 PORSCH (**20**) proposed the theory that the two cell groups in the opposite ends of the angiosperm embryo sac are both to be interpreted as archegonia. The synergids are thought to correspond to two neck canal cells, and the upper polar nucleus to the ventral canal cell of the archegonium. According to this theory the embryo sac of Helosis consists of only one archegonium, the other having been suppressed. The behavior of *Smilacina stellata* perhaps supports this theory, in so far as it shows the equivalent origin of the two groups of nuclei which PORSCH interprets as archegonia.

Miss PACE (**19**) has reported that Cypripedium forms a four-celled embryo sac by two divisions of the lower of two "megaspores." This embryo sac may be interpreted by PORSCH's theory to be a single archegonium.

WENT (**28**), in a recent article on the Podostemaceae, reports that

in Oenone and Mourera a four-celled embryo sac is formed, similar to that in Helosis. The mother cell following synapsis divides to form two daughter cells, the upper degenerating. The lower daughter cell (which he calls the "megaspore") divides, and the innermost nucleus shrinks immediately to a shapeless mass of chromatin that is visible for a long time. This inner nucleus, though one of the first four nuclei formed by the division of the embryo sac mother cell, is interpreted by WENT as a nucleus of the embryo sac and not a spore. The nucleus remaining from the second division divides twice to form the four-celled embryo sac. The lower nucleus of this embryo sac degenerates, leaving only the three nuclei which form the egg apparatus. WENT notes that his results support the theory of PORSCH, except that the ventral canal cell is on the wrong side of the egg.

ERNST (**10**) has expressed the opinion that the 16-nucleate embryo sac of Gunnera, as described by him, consists of two of the archegonia of PORSCH in the chalazal end of the embryo sac and one in the micropylar end. Four nuclei in the central region of the embryo sac fail to form an archegonium, and unite with a polar nucleus from each archegonium to form the endosperm nucleus.

STEPHENS (**23**), in a preliminary note on certain Penaeaceae, reports that the sixteen nuclei of the embryo sac become divided into four groups, which lie at some distance from one another against the wall of the embryo sac. Three nuclei out of each group of four organize what appears to be an egg apparatus, while one nucleus from each group acts as a polar nucleus, and the four unite to form the endosperm nucleus. On PORSCH'S theory these four groups would represent four archegonia.

These examples seem to indicate a tendency of the nuclei of the angiosperm embryo sac to form groups of four. The evidence in favor of the archegoniate character of these groups, however, seems to me to be insufficient as yet. The relationship between the angiosperms and the gymnosperms is so remote that a comparison of the embryo sacs of the two groups with a view to homologizing the groups of nuclei associated with the egg is very difficult.

COULTER (**8**) further defends the idea that the first four nuclei of the embryo sac of the lily are megaspores, or "at least their nuclei."

His evidence lies in the fact of their being the product of the two reduction divisions. Hence they must be "megaspore nuclei, to be recognized as such by their cytological history and structure." The 16-nucleate embryo sacs of *Peperomia pellucida* are considered as resulting from two divisions of each of these megaspore nuclei, and the 8- and 16-nucleate embryo sacs, which JOHNSON reports as occurring in *Peperomia hispidula*, are regarded as formed by one division of each of the four megaspore nuclei to form the 8-nucleate embryo sac, and by two divisions of each to form the 16-nucleate embryo sac.

COULTER is inclined to regard it as a fundamental law that the angiosperm embryo sac is formed from the mother cell by never more than five nuclear divisions, the reduction divisions and three divisions of a megaspore. He concludes that the large number of nuclei in the embryo sacs of Peperomia and Pandanus may originate by the participation of more than one spore in their organization. This implies, further, that any embryo sac formed from one spore which has sixteen or more nuclei can be considered as relatively primitive, since it would require more than five nuclear divisions to produce it from the mother cell.

I do not see why the cases of the proliferation of antipodal nuclei should not be given more weight in the evidence. There is certainly an abundance of cases among the grasses and in the Ranunculaceae, as well as in other families, in which the innermost nuclei of the embryo sac are the product of more than five divisions previous to fertilization. It seems doubtful whether the embryo sac with more than eight nuclei can be explained on any such simple hypothesis, and it is to be remembered that, while there is apparently a physiological necessity back of the double division, there is nothing, as STRASBURGER has noted, in the phylogeny of the angiosperms which would explain or give special significance to a fivefold division.

BROWN (1) reports that evanescent cell walls are formed separating the first four nuclei of the embryo sac of *Peperomia Sintensii* and *P. arifolia*, in both of which the mother cell develops directly into the embryo sac. Though evidently convinced that in Peperomia these first four nuclei are megaspores, BROWN seems to object to the adoption of this explanation for the lily type of embryo sac in general.

Referring to the number of divisions which archesporial cells undergo to form the mother cell, he says: "Since we can trace the reduction of these divisions until, among the angiosperms, the archesporial cell may, without dividing, form one megaspore mother cell, it does not seem reasonable to suppose that the divisions of the mother cell to form four megaspores may not also be left out, and the mother cell function directly as a megaspore." Any analogy, however, based upon the archesporial cell, has against it that this latter cell is a doubtful morphological unit.

It cannot be maintained that any morphological interpretation yet proposed satisfactorily explains all the diverse types of embryo sac formation which have been described. That there are so many variations in important particulars gives ground for the expectation that further study may fill up many gaps in our current interpretations.

It is plain that within the group of the Convallariaceae there are types which go far toward explaining the origin of the old and familiar lily type of embryo sac. It is certainly plain that in *Smilacina stellata* four megaspores are formed, which are unmistakably separated by cell walls and subsequently recombine to form the first four nuclei of the embryo sac.

All the evidence favors the view that the first four nuclei of the lily embryo sac are morphologically, as well as from the standpoint of the reduction divisions, to be interpreted as megaspore nuclei.

SUMMARY

1. The mother cell of *Smilacina stellata* divides twice to form four nuclei, which are separated by walls to form four megaspores.

2. The division walls and plasma membranes which separate the four nuclei are absorbed, so that the four reduction nuclei occupy a common cell cavity.

3. Each of these four nuclei divides again, and the resulting eight nuclei organize to form the embryo sac.

4. It is plain from these facts that we have four individual megaspore cells combining to form one embryo sac or gametophyte in *Smilacina stellata*.

5. It is thus strongly suggested that in the embryo sac of the lilies the first four nuclei are morphologically megaspores.

6. In *Smilacina racemosa* the outer daughter cell of the heterotypic division develops into the embryo sac, although the long persistence of the two nuclei formed by the division of the inner heterotypic nucleus might suggest that they should be considered a part of the embryo sac. *Smilacina racemosa* shows temporary cell division at the close of the homeotypic division, agreeing in this respect with *Smilacina stellata*.

I wish to express my obligations to Professor H. D. DENSMORE, in whose laboratory at Beloit College this investigation has been largely carried on. I am also indebted to Professor R. A. HARPER for his suggestions and criticisms during the preparation of the manuscript.

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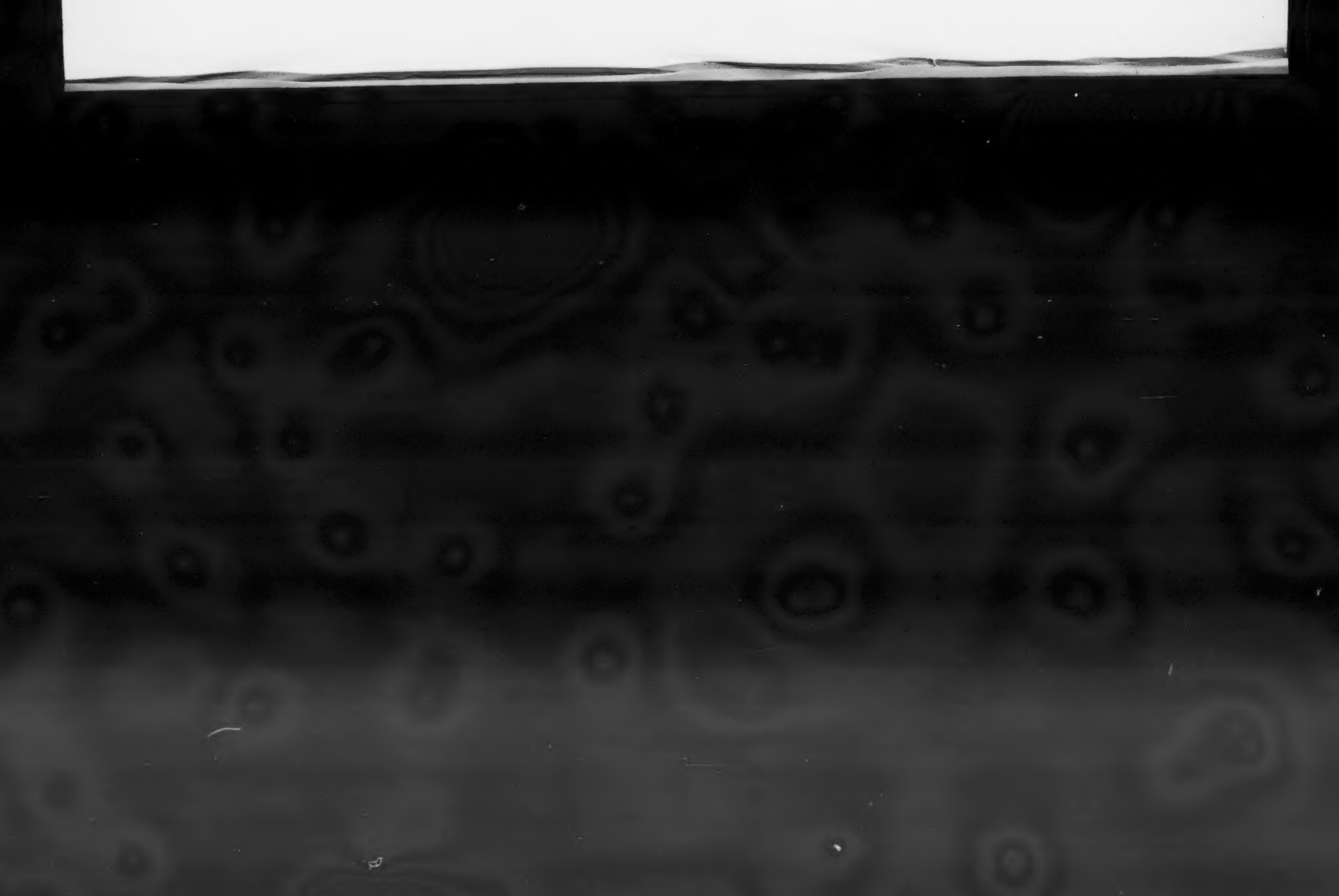
EXPLANATION OF PLATE XV

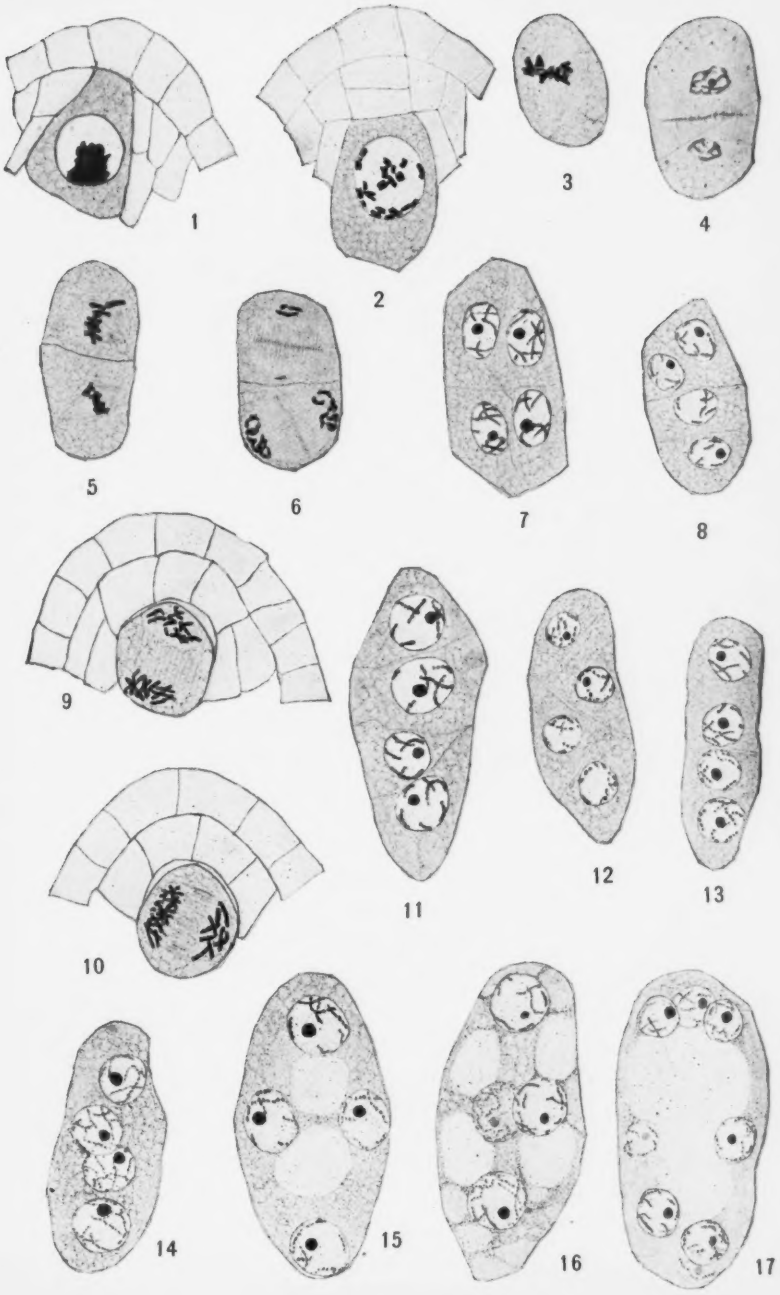
All figures were drawn with the aid of a camera lucida. The magnification in all the drawings is 670 diameters. The micropylar end is upward in each drawing.

FIG. 1.—Mother cell in synapsis stage; without tapetal cell.

FIG. 2.—Mother cell in prophase stage of first division; two cell layers between the epidermis and the mother cell.

FIG. 3.—Early metaphase stage of the mother cell, showing thick double chromosomes characteristic of first reduction division.





McALLISTER on SMILACINA

FIG. 4.—Formation of cell plate and daughter nuclei at the close of first division of mother cell.

FIG. 5.—Early metaphase stage of homotypic division; axes of spindles oblique.

FIG. 6.—Daughter nuclei and cell plates forming at the close of second division; outer daughter cell dividing transversely and inner one longitudinally.

FIG. 7.—Nuclei of second division fully formed, arranged bilaterally; division membranes of second division do not reach outside walls.

FIG. 8.—The outer daughter cell has divided longitudinally and the inner one transversely.

FIGS. 9, 10.—Two successive sections of the same nucellus, showing the division of an approximately spherical mother cell; heterotypic division longitudinal; one daughter cell in the homeotypic division dividing longitudinally and the other transversely.

FIG. 11.—Row of four very large megaspores, with the division walls very indefinite.

FIG. 12.—Commonest arrangement of megaspores, in which first division is oblique and second at right angles to first; walls have disappeared but plasma membranes still persist.

FIG. 13.—Four megaspores; a cleft between two middle nuclei is all that remains of division membranes of cells.

FIG. 14.—Four megaspores, with no traces of cell walls, forming four-celled stage of embryo sac.

FIGS. 15, 16.—Stages in the vacuolation of the four-celled embryo sac.

FIG. 17.—Eight-celled embryo sac, showing micropylar and antipodal groups of nuclei, the two polar nuclei already separated out.

A STUDY OF PIÑON PINE

F. J. PHILLIPS

GENERAL DISTRIBUTION

No other tree species of the southern portion of the Rocky Mountain region presents more difficult problems in maintaining and reproducing the natural stands than does the piñon pine (*Pinus edulis*). It ranges from northern Mexico to eastern Utah, and Colorado Springs, Colorado. In an east-and-west direction it extends from the hills of western Texas to California. Along the northern and eastern borders of its range it is shrublike and of botanical importance only. In southern Colorado, Arizona, and New Mexico, it has a great economic and silvicultural importance, which will steadily decrease unless measures are taken to prevent excessive utilization.

It is commonly found in mixture with the one-seeded juniper (*Juniperus monosperma*) in the northern part of its range and with the alligator juniper (*Juniperus pachyphloea*) and one-seeded juniper in the south. Throughout its distribution it is associated with western yellow pine (*Pinus ponderosa*) and the scrub oaks (*Quercus Gambelii* and *Quercus acuminata*), often forming with these species a transition belt between stands of juniper and western yellow pine. Occasionally it is found with stunted Douglas fir (*Pseudotsuga taxifolia*). In association with the junipers, it forms the distinct woodland type so characteristic of New Mexico and Arizona, which in this region covers a more extensive area than any other forest type, and in which the piñon is decidedly the most important tree. It is occasionally seen in pure stands over small areas, but this is rare.

LOCAL OCCURRENCE

The tree thrives best at a general elevation of 1650 to 2350^{m} (5400 to 7700 feet) on moderate to steep mountain slopes and over broad, level, or sloping mesas. Small isolated specimens were found up to an elevation of 2600 and 2750^{m} (8500 and 9000 feet), while occa-

sional specimens may be found even higher than this. The best stands are found on coarse gravel, gravelly loam, or a coarse sand, of 1.5[m] (5 feet) or more in depth, on which humus and ground cover are almost entirely lacking. The species often occurs on rocky areas, where the soil is only 15 to 30[cm] (6–12[in]) in depth, and frequently it is found growing in rock crevices. It is one of the first trees to gain a foothold on the lava overflows which are known throughout the southwest as *mal pais*. This rock in its disintegrated form supports fair tree growth, but even before disintegration has progressed very far, the junipers and piñon may be found encroaching upon it.

Another encroachment form of the piñon is to be found on small mounds which rise 0.6 to 3[m] (2 to 10 feet) above the general level of the desert-like tableland at approximately 1500[m] (5000 feet) elevation. On such islands as these, the piñon and one-seeded juniper take possession and maintain a limited growth. The same feature is noted at the bases of the hill and mountain slopes which bound these tablelands. This remarkably distinct tension line seems to be due to a greater soil porosity, less grass growth, and a smaller alkali content, which are manifest in slightly higher elevations. The distribution of these trees on such small mounds and limited in such a distinctive manner presents an ecological problem for future investigation.

On slopes where site conditions are favorable for western yellow pine, the piñon usually occupies the south and west aspects. Where conditions become less favorable, it occupies the north and east slopes, while the south and west slopes are bare or nearly so. This ability to stand poor conditions is also shown on a large number of mountain slopes ranging from 2830 to 3135[m] (6000 to 7000 feet) in elevation, where scattering Douglas fir, of scrubby growth and badly affected with witch's broom, is found in the cañons; western yellow pine on the middle slopes; and piñon on the ridges and upper slopes, where the soil is scant and the soil moisture low.

A distinctive peculiarity was observed between Servilleta and Taos, New Mexico, in an open stand of the species in which approximately two-thirds of the trees have constricted bases at the surface of the ground. This constriction amounted to an average of 19[mm] (0.75[in])

in radius, but was occasionally noted where it amounted to 38^{mm} (1.5^{in}). Such a constriction is often seen on individual trees in nearly any stand, but in no other case was it found to be a stand as characteristic as it was near Servilleta.

Piñon is also resistant to severe climatic conditions, since it will succeed over severely exposed slopes where the average annual precipitation is less than 33^{cm} (13^{in}) and where evaporation and transpiration are high because of the semi-arid climate, the large amount of sunshine, and the prevalence of winds. In this respect it is undoubtedly the most resistant pine in the southwest. However, it prefers a slightly greater precipitation and areas less exposed to the wind. An example of the unfavorable influence of strong winds and a close-textured soil was noted in the vicinity of Fort Stanton, New Mexico, where a level plateau of nearly 8^{km} (5 miles) in length did not support a single tree, while similar plateaus on all sides, with less wind sweep and a coarser soil, showed luxuriant growth of both the piñon and the juniper. The tree does not live as long as the junipers, and in general is less resistant to unfavorable climatic conditions. In the drought which occurred in New Mexico from 1889 to 1904, piñon suffered considerably more than the junipers. Many mixed stands were observed in New Mexico and southern Colorado in which 75 to 95 per cent. of all dead trees were piñon. In the frost which occurred in April, 1907, piñon was affected, while the junipers resisted practically all injury. In the wet freezing snow of October, 1906, which caused immense damage to the forests of the southwest, fewer branches were broken from the piñon than from the brittle junipers.

The tree is also more resistant to disease than most of the conifers with which it associates. It is much less affected by the so-called false mistletoe (Razoumofskya) than is the western yellow pine and the junipers. It has fewer insect enemies than the western yellow pine, and is not affected by the witch's broom as is often the case with Douglas fir in the southwest.

TOLERANCE AND FORM

Piñon is distinctly an intolerant tree. During its seedling stage it prefers a moderate shade, and hence reproduces best under the

shade of older trees. After the seedling stage is passed it prefers the open, and is one of the most intolerant of forest trees. This gives an orchard-like appearance to most stands of this species. Occasionally stands of 0.7 density were noted, although few stands have more than 0.6 density.

On the best sites the trees reach a maximum height of 12 to 13.7^{m} (40 to 45 feet) and a diameter of 60 to 75^{cm} (2 to 2.5 feet) at breast height, but ordinarily the mature individuals range from 3 to 10.5^{m} (10 to 35 feet) in height and from 15 to 45^{cm} (0.5 to 1.5 feet) in diameter. A difference in development was apparent on different sites. On exposed sites the tree is globular, very scraggly when mature, and has little or no clear length. On favorable sites trees in the open have a very short clear length and a fairly regular globular or egg-shaped crown. If grown in stands, the trees have a greater clear length and a flat or vase-shaped crown. Young trees on favorable sites are conical or globular in shape and usually very regular in form.

On the most exposed sites, shrublike trees were found which were fifty to eighty years old, and only 1.8 to 3^{m} (6 to 10 feet) in height, with a crown diameter reaching a maximum of two to four times the height of the tree. On such trees it was impossible to distinguish the leader from the branches, and the general appearance of the tree was much like that of the dwarf mountain pine (*Pinus monticola*). The foliage is more densely clustered on these dwarf trees than it is on trees in the open, with shorter and apparently thicker leaves. Practically all trees, whether growing on poor or good sites, are characterized by dead and half-dead branches, which are retained on the tree for several years. This is characteristic of nearly all species in the southwest and is due to the small amount of growth that is made, the necessity of retaining only a small amount of living tissue, and the dry nature of the climate, which allows the retention of dead branches for a longer period than would a moist climate. In exceptional stands, such as occur to the west of Servilleta, New Mexico, where a clear length of 4.5 to 7.6^{m} (15 to 25 feet) is not exceptional, the branches are shed largely because the density of stand prevents the formation of as large branches as are found in those trees which enjoy full sunlight.

WOOD

Piñon wood is moderately heavy for the pines. It is used extensively for fuel and has been limitedly used for fence posts, telephone poles, corral posts, mine lagging, railroad ties, charcoal, and inferior lumber. Some authorities have recommended its use for fence posts, but this is to be seriously questioned as it has little durability in contact with the soil, and even the natives are discarding it for such use. It may be rendered valuable, however, by the use of preservatives. The tree is remarkable in its fuel value, and its use for such a purpose should be greatly encouraged. It is a common practice to cut branches or trees after they have been dead about two years. If cut before this time, the wood has not seasoned sufficiently to burn readily. If cut after this time, it has usually deteriorated to some extent. As a hearth fuel, it is not surpassed by another conifer and by only few hardwoods. It starts to burn readily, retains fire for a considerable length of time, gives a large amount of heat, and does not throw sparks. Since open fires are very common in this region, this wood serves an excellent purpose. Sample acres which have been clear cut have given a yield of 180 to $360^{\text{cu m}}$ per hectare (20 to 40 cords per acre), while extensive stands have averaged 90 to $108^{\text{cu m}}$ (10 to 12 cords).

FRUIT

The young cones are dark red and occur in elongated clusters. The pistillate form is easily distinguished by short stalks. Both sorts are very plentiful in seed years, but are scarce during other years. The mature cone is short, top-shaped, 19 to 50^{mm} (0.75 to 2^{in}) long and often as broad as long. The cones open on the tree and are covered by a large amount of free resin, which makes them difficult to handle. They often occur on trees only 0.9 to 1.2^{mm} (3 to 4 feet) in height, which are ten to twenty years old, but the best crops are borne on mature trees which produce 35 to 280^{l} (1 to 8 bushels) of cones; each cone contains two to thirty seeds, with an average of ten to twenty seeds. The trees have been known to yield 336^{kg} of seed per hectare (300 pounds per acre), while a much larger area has been known to produce an average of 73^{kg} per hectare (65 pounds per acre).

Seed years usually occur at five-year intervals, but have been reported at shorter intervals than this. The seed is well rounded at the base, tapering with prominent ridges to an acute point. It is usually dark brown on the lower side, with more or less mottled orange yellow on the upper side, 9 to 12.5^{mm} (0.375 to 0.5^{in}) long, 6.5 to 9^{mm} (0.25 to 0.375^{in}) broad, with a thin shell which cracks most easily along the line of the most prominent ridge. The seed wings are about one-half the length of the seed, easily detached, and of no practical use in seed distribution. The seeds usually have a high percentage of infertility, which varies from 5 to 20 per cent., but in one case went as high as 85 per cent. Poor seeds are often lighter in color than good seeds. Germination power is lost very readily, which necessitates special storing when they are to be used for artificial planting, and good site-conditions when the stands are to be reproduced naturally. It is a matter of note that the seeds from the northern portion of the range are usually considered better than those from the south. Five samples collected in various localities gave the following results:

No. per pound (453.6^{gm})	Percentage viable; knife test	Percentage viable; water test	Percentage viable; in greenhouse	Percentage viable; in open	Where collected
2510	87.2	84.0	82.2	75.6	Ft. Bayard, N. M.
2215	89.1	86.6	80.3	69.2	Tres Piedras, N. M.
1810	91.2	86.0	78.1	70.4	Ft. Garland, Col.
1950	92.7	88.5	81.3	71.0	Ft. Garland, Col.
1520	99.2	97.1	96.4	90.3	Lincoln, N. M.

Weevils sometimes affect the seed before the cones open. Birds and rodents eat the seed extensively, and stores are made by mountain rats which were found to contain a maximum of 35 to 70[1] (1 to 2 bushels) of clean seed. Ants are known to eat seed, especially at lower levels. In the early days, the Indians and Mexicans used the piñon as a staple article of food. At present, it is gathered in immense quantities and sold as a delicacy. It is eaten most extensively in and about the region where the tree grows naturally, but large amounts are being sold at fruit stands throughout most of the United States. To prevent the seeds from spoiling and to retain flavor, they are usually baked immediately after being gathered.

Most of the seeds are collected by Mexican women and children, who usually spread a sheet or blanket on the ground and then shake or pound the tree and its branches until the seeds fall from the open cone. Later in the season, the seeds are picked up by hand from the ground beneath the trees. In the best part of the seed harvest, enough are gathered by single families to be sold by the grain bag full or the wagon load. Since the Mexicans take almost no precautions against the spreading of smallpox, it is said that the worst ravages of the disease occur during a seed year of the piñon. Single dealers have been reported as having bought 9000 to 21,500[kg] (20,000 to 50,000 pounds). The delicate flavor of the seed makes it a favorite, and an extensive market is being rapidly developed for it. During seed years the native collectors sell it at the rate of five to fifteen cents per pound, according to the ease of collecting the seed and the proximity of the market, while dealers in many of our cities sell the seed at a rate of forty to sixty cents per pound.

REPRODUCTION

Natural reproduction is limited because of the infrequency of seed years, unfavorable climatic conditions, infertility of seed, rapidity with which the seed loses its germination power, loss of seed eaten by rodents, birds, and man, and unfavorable site-conditions. Grazing interests are also a factor in limiting the reproduction of the species, since sheep, cattle, and goats are grazed throughout its entire distribution. It is apparent to even the casual observer that extremely large areas are not reproducing themselves, yet owing to the difficulties of site and the methods by which the tree may be reproduced, the problem of reproduction is an extremely difficult one, and one for which, at the present time, no adequate solution can be offered.

FUTURE MANAGEMENT

From the nature of the stand in the southwest, it is apparent that clear cutting would not be an advisable system, because of the exposure of the site and the difficulties of restoring the stand. On the other hand, the large amount of seed consumed by man and other agencies makes natural seeding exceedingly difficult, and even though grazing and fire are entirely eliminated, it is doubtful if satisfactory reproduc-

tion will be secured in even a bare majority of sites. Until the problem of reproduction is more thoroughly worked out, the policy should be to remove only the dead and dying piñon trees for fuel, thus allowing a careful management without encroaching seriously upon the natural stands as is being done at the present time. It would seem from the nature of the site that the stand could be made to succeed best by the selection system, consisting of the removal of the dying trees. The sale of this fuel with that of a large portion of the seed should furnish a moderate income. This production would be low, as contrasted with high-type coniferous forests in other regions, but when consideration is given to the value of this species for fuel and seed, the question of immediate returns is a minor one.

UNIVERSITY OF NEBRASKA

BRIEFER ARTICLES

ON THE DEMONSTRATION OF THE FORMATION OF STARCH IN LEAVES

For a qualitative demonstration of photosynthesis in starch-forming leaves it is advantageous to know the time in darkness required for the disappearance of accumulated starch, and the time in light required for its subsequent demonstrable formation. No general rule can be given for either, since the time required varies widely for the different species. Therefore, in continuation of the series of studies carried on in the laboratory of plant physiology of Smith College on the physiological constants of the educationally useful plants,[1] I have tried to determine these data for such plants, and also, by comparison, the best plants for the purpose. Throughout this paper I have used the expression "disappearance of starch" rather than "translocation." In a general way the processes, of course, correspond, yet the term "translocation" implies the removal of the starch from the leaf, while here we are dealing only with its disappearance as starch.

The method employed in the present study was as follows: Five actively growing plants of each species, always after a bright day and between 4 and 5 o'clock, were put in a dark room having a steady temperature of 18°–22° C. as recorded by a thermograph. Twice a day, about 9 A. M. and 2 P. M., leaves were tested for starch by SACHS's iodine method, and the time when all starch had disappeared was noted. No attempt was made to find the exact hour when the leaves were empty. This would necessitate testing them every hour during the night as well as during the day, and for the purpose of the present study, the results would be of little value. In the following table, therefore, the time in darkness required to empty the leaves of starch is given in night and day periods rather than in hours. For the iodin test the leaves were first boiled 1 minute to swell the starch, were blanched in warm alcohol, were put in water a few minutes to remove the alcohol and soften the tissues, and were then immersed in a solution of iodin. The solution used was 5^{gm} potassium iodid, 1^{cm} iodin, 10^{cc} water, to which, when dissolved, water was added to make 1 liter of solution.

Thus was the time of disappearance of starch determined with sufficient accuracy for all practical purposes. To determine the time required

[1] BOT. GAZETTE **40**:302. 1905; **45**:50. 1908; **45**:254. 1908; **46**:50. 1908; **46**:221. 1908.

for starch formation, it was found best to use some type of screen such that a sharp contrast would show between the light and dark parts; and for this purpose light-screen boxes were used. These boxes have been fully described by Professor GANONG in the BOTANICAL GAZETTE.[2] Briefly, they are small boxes made of white paper blackened inside, with a network of threads across the top to support the leaf, and holes near the bottom to allow the air to pass through freely. A glass plate covered with tinfoil having a pattern cut in it is held closely against the network by a wire spring. When in use the leaf is held between the glass plate and the network. The principal advantage of these screens is that while excluding all light, they allow nearly the normal access of carbon dioxid to the leaf. The caution, by the way, against using screens which cut off all carbon dioxid as well as the light has been made several times in recent years, but some of the new elementary textbooks are still copying the old and erroneous method of putting cork or tinfoil on both sides of the leaf. Several light-screen boxes were attached to the leaves which had previously been emptied of starch, and the plants were placed in strong diffuse light. Leaves were then taken off and tested for starch at 10-minute intervals. In order to compensate the effects of individual peculiarities, 5 plants of each species were tested. The results given in the table are for full-grown (except in the three cases noted), but not mature, leaves on actively growing plants, in pots, in a greenhouse. In the first column is given the time in darkness required to empty the leaves of starch; in the second, the time in diffuse light required to make enough starch to show a pale but clearly defined figure with the iodin test; in the third, the time required to show a sharply defined, dark figure; while in the fourth is given the time required for the iodin to produce its full effect.

The best leaves, obviously, for this study are those in which photosynthesis is most active, from which starch disappears most rapidly in darkness, from which the chlorophyll can be extracted quickly leaving the leaf white, and which give the iodin reaction quickly. As shown by the accompanying table, these are *Pelargonium hortorum zonale*, *Fuchsia speciosa*, *Senecio mikanioides*, *Impatiens Sultani*, and young plants of *Helianthus annuus*, *Ricinus communis*, *Phaseolus vulgaris*, *Zea Mais*, and *Cucurbita Pepo*.

On the other hand, some leaves are not good for this study. *Begonia palmata*, *Oxalis Bowiei*, and *Pelargonium peltatum*, when boiled to swell the starch, partially disintegrate, so that the figure does not show clearly with the iodin test. It is possible, of course, to apply the iodin test without previous boiling of the leaf, but it takes 24 to 48 hours according to the

[2] BOT. GAZETTE 43:277. 1907.

age of the leaf; besides, these leaves turn brownish yellow on the application of iodin and therefore do not show clearly the reaction with starch. *Ficus elastica* requires more than one day to make a perceptible amount. Only very young leaves of *Primula obconica*, *Primula sinensis*, and *Cineraria cruenta* can be emptied of starch; being stemless plants, they probably use the older leaves for its storage. Young plants of *Pelargonium domesti-*

Name of plant	Disappearance of starch in darkness (T. 18°–22° C.)		Formation of starch in light (T. 20°–25°C.)		Iodin test
			Perceptible fig.	Good fig.	
	nights	and days	minutes	minutes	minutes
Abutilon	2	1	30	120	5–10
Begonia coccinea	3	3	60	240	10
Brassica oleracea	1	0	20	50	5
Cineraria cruenta (young ls)	5	4	45	180	30
Coleus Blumei	2	1	30	50	3–8
Cucurbita Pepo	1	0	15	50	4–15
Euphorbia pulcherrima	2	1	60	240	20
Fuchsia speciosa	1	0	45	90	15–25
Helianthus annuus	1	0	30	120	5
Heliotropium peruvianum	1	0	45	120	5–10
Impatiens Sultani	1	0	30	120	5
Lupinus albus	1	0	60	240	3
Oxalis Bowiei	1	0	45	240	10–30
Pelargonium domesticum	2	1	50	240	40
Pelargonium peltatum	3	3	50	270	60
Pelargonium hortorum × zonale	2	1	20	50	10–15
Phaseolus vulgaris	1	0	20	90	5
Primula obconica (young ls)	5	4	120	240	5–10
Primula sinensis (young ls)	4	3	45	120	10
Raphanus sativus	1	0	35	60	3
Ricinus communis	1	0	20	60	5–15
Salvia involucrata	2	1	90	120	4
Salvia splendens	3	2	30	60	1–5
Senecio mikanioides	1	0	20	50	5
Senecio Petasitis	3	2	30	180	25
Tropaeolum majus	2	1	50	90	1
Vicia Faba	1	0	60	240	10
Zea Mais	3	2	30	120	5

cum empty their leaves of starch in about 40 hours, but older plants are unsatisfactory, because, even after 4 days in darkness, there are still spots of starch in the mesophyll. All these plants, except *Oxalis Bowiei*, and the young plants of *Pelargonium domesticum*, require 3 to 5 days in darkness —too long a time for the subsequent good of the plant.

Prolonged darkness produces two distinct deleterious results. First, some plants, as Heliotropium and Impatiens, after 3 or 4 days in darkness, drop their leaves, owing to some derangement of their mechanism, the

cause of which I have not investigated. Also, Tropaeolum leaves soon turn yellow in darkness. The second injurious result, and the most important in this study, occurs in nearly all plants. Some of the photosynthate is of course being constantly used in growth or for storage in the stem, and since the plant can make no more in darkness, the percentage of sugar in the cell-sap decreases more and more by diffusion into the stem. Then, as PFEFFER has shown,[3] when the plant is brought into the light no starch is deposited until this percentage of sugar is repaired. The following example is typical: Two plants of *Fuchsia speciosa*, apparently alike, were put in the light at the same time, after having been in darkness, the one 64 hours, the other 16 hours. Halves of leaves tested showed no starch. After 2 hours in the light, the other halves on the first plant showed much less starch than those on the second plant. To obtain the most rapid formation of starch, therefore, it is important that the plant should be kept in darkness only long enough just to cause the disappearance of starch.

The disappearance of starch is not always even. In Coleus, *Primula verticillata*, *Primula obconica*, and *Fuchsia speciosa*, the base of the leaf is emptied of starch before the tip. This agrees with what SACHS found in some other leaves.[4] So far as I have tested them, I have found this to be true only of ovate or oblong leaves. This may be correlated with the greater abundance of stomata near the base of the leaf. In round leaves like *Pelargonium zonale* and Tropaeolum, the starch seems to disappear evenly from all parts. The starch disappears from the young leaves on a plant before it does from those which are mature.

The effects of temperature on the amount of starch present are especially important. In several of my experiments, leaves of *Fuchsia speciosa*, *Euphorbia pulcherrima*, and *Pelargonium zonale* which were kept in direct sunlight 4 hours showed very little starch, while leaves on plants in diffuse light, at the end of 4 hours were full of starch. *Fuchsia speciosa* after being in diffuse light at 28°–31° C. for 3 hours showed only a trace of starch, while other leaves in 3 hours at 18°–20° C. appeared black with starch, with the iodin test. A comparison of these two sets of experiments shows that the small amount of starch present in leaves in direct sunlight is undoubtedly connected with their high temperature. In order to get the best results, therefore, from experiments in the formation of starch in the leaves of potted greenhouse plants, it is necessary to keep the plants at a temperature not exceeding 22° C., and to insure this it is well to keep them in diffuse rather than in direct sunlight. It will be of interest to compare these results with those obtained by SACHS for outdoor plants, as described in his classical

3 Physiology 1:321. 4 Ges. Abhandl. 360.

paper.[5] He found that the rate of the disappearance of starch from the leaves increases with the temperature. When the nights were very warm, some plants (Nicotiana, Phaseolus, Juglans, and others) completely emptied their leaves of starch in one night. But after cool nights, 6°–9° C., there was no perceptible loss of starch in some, and the disappearance was incomplete in others. SACHS found also that the amount of starch present at any time of the day is affected by temperature. At a temperature of 20°–25° C. the quantity of starch in the leaves increased steadily from morning until evening. But on hot afternoons at a temperature of 30°–35° C. the leaves of Helianthus contained less starch than in the morning at 8 o'clock. The reason for this phenomenon is found in the fact that translocation from the leaf into the stem increases with rising temperature more rapidly than photosynthesis. All of these considerations emphasize the important precaution that to obtain the best starch formation in leaves, the temperature should not be permitted to rise above 20°–22° C., which is apparently the optimum for this process, as it is for the best general health of such plants as are used in these studies.

Also it must be remembered that plants cannot give good results in this or any physiological experiment, when suffering from previous starvation, as in the case of pot-bound plants or those which have been kept in darkness for a long time; when suffering from over-stimulation from high feeding, or from being kept at too high temperatures; and when they have passed their grand period of growth. As a rule plants, and particularly annuals, are in their best condition just before flowering.—SOPHIA ECKERSON, *Smith College.*

THE MORPHOLOGY OF *RUPPIA MARITIMA*—A CRITICISM[6]

In section D, "Function of the root," GRAVES (p. 113) has wandered beyond the natural limits of his paper as a morphological study, and while the propriety of this is perhaps questionable, the basis for this criticism is that this section D contains a statement which I think is an unwarranted misinterpretation of some of my own writings, and also a statement that would leave most readers misinformed. I wish first to consider the following: "On the other hand, POND's experiments[7] fail to show conclusively whether or not water and dissolved salts are absorbed by the part of the plant above

[5] Arbeit. Bot. Inst. Würzburg **3**:1 ff., as cited in Ges. Abhandl. 354–387.

[6] GRAVES, A. B., The morphology of *Ruppia maritima*. Trans. Conn. Acad. Arts & Sciences **14**:59–170. 1908.

[7] POND, RAYMOND H., The biological relation of aquatic plants to the substratum. U. S. Fish Commission Report **1903**:483–526. 1905.

the soil." Being somewhat uncertain as to the intended meaning of this statement, I have learned by correspondence that GRAVES believes that in my paper I have committed myself to the notion (in the absence of conclusive evidence) that the larger submerged and rooting water plants derive their mineral food exclusively from the soil, and that there is no absorption of mineral food by organs other than the roots. Such a notion I have never held and do not consider my paper as warranting or encouraging such an interpretation. My efforts to force certain species to live and grow in nutrient solutions and without a substratum were not continued to a satisfactory conclusion, and for that reason I remained noncommittal on the possibility of absorption by the other organs of the plant.

My general conclusion was that certain species which were tried were found to be dependent upon their rooting in the soil for optimum growth and cannot survive a single season if denied a substratum of soil.

Whatever the absorption by the organs other than the roots amounts to (in the species tried), it is so small that the species would probably become extinct if forced to depend upon it exclusively under otherwise natural conditions.

Looking at section D as a whole we find that every paragraph is general in its scope and treatment. GRAVES enumerates four reasons for not placing too much emphasis "on the absorbing capacity of the root." One of the reasons is "the total lack of branches and slenderness of the roots." I think any reader would infer that submerged plants have slender roots without branches. So far as I know the statement is correct so far as the "slenderness" is concerned but in my paper (*l. c.*) there is a section on factors influencing the development of lateral roots

Speculation as to the relative importance of absorption by the roots of submerged plants as compared with that in the case of land plants is of little value. I refer particularly to the statement by GRAVES as follows: "In brief, the absorption carried on by the roots of submerged plants and the importance of this function in the economy of the plant is much greater than is implied by SCHENCK—but, on account of the peculiar environmental conditions of submerged plants, it can never equal in importance the absorption of the roots of land plants."

If the submerged species are as dependent upon rooting in the soil as my results show, the so-called absorptive function of the root is a vital necessity and it certainly is not more than this for land plants.—RAYMOND H. POND.

CURRENT LITERATURE

BOOK REVIEWS

Bacteriology, general and special

Although bacteriology in the past decade or more has developed to such an extent as to warrant being considered more broadly than merely as an *Anhang* to medicine, yet the texts prepared for the use of biological students have been written, in the main, from a comparatively restricted point of view, usually being designed for medical students. Naturally, the limitations of time demand that the student in technical courses, such as medicine, shall receive specific instruction in the subject-matter relating to the activities of those organisms that are of especial moment to him; but in our larger universities the time is ripe for the presentation of the subject of bacteriology from the standpoint of the student of general science. A course of training in general bacteriology is as essential in supplementing a general course in biology as is general chemistry. The works of DE BARY and FISCHER served as a suitable foundation in their day for the treatment of bacteria from the biologic viewpoint, and there is great need that such works be brought down to date.

Dr. JORDAN, the author of a recent textbook of general bacteriology, has had an unusual opportunity, by training and position, to develop a strong course in this subject; yet one cannot help feeling that even in a university atmosphere, the needs of the medical student were uppermost in his mind in the preparation of his book. And this impression prevails in spite of the fact that the volume purports to cover the general field, and attempts to present the "perspective of the subject, with emphasis on general rather than on special questions." A casual inspection of the book gives the impression that it is in the main medical, but a more careful analysis shows that much more satisfactory treatment of the non-medical phases is given than is generally the case. About 70 of the 540 pages of text are devoted to the morphology and physiology of the bacteria. The addition of chapters on organisms of the soil, air, and water, as well as of milk and its products, and on bacterial plant diseases, widens the scope of the work, but it is very evident that the treatment in these special chapters is no so well balanced as is the presentation of the animal pathogens. Indeed, the work, as a whole, is so largely medical in its aspects that we feel that there is yet to be written a text on general bacteriology that will fairly present these organisms from the biological point of view. For example, it would seem that a subject of such biological and historical importance as fermentation should

[1] JORDAN, EDWIN O., A textbook of general bacteriology. 8vo. pp. 557. *figs. 163.* Philadelphia: W. B. Saunders Co. 1908. $3.00.

receive adequate treatment. Bacteriology will never attain its true position as a member of the sisterhood of biological sciences until some one of its devotees will sacrifice his time to prepare a comprehensive text that presents the bacteria in their relations as living things, rather than as capable of affecting prejudicially or otherwise man and beast. Special fields may well receive special treatment, with such summary of general matter as may be absolutely necessary to enable the special class of students to handle the subject.

While the volume fails somewhat to meet our anticipations and is hardly adequate from the biologic point of view, the admirable presentation of the medical part of the volume shows careful work. The addition of chapters on the pathogenic Trichomycetes, Blastomycetes, and Hyphomycetes, as well as the disease-producing protozoa and diseases of unknown etiology, will be helpful to the medical student in correlating many of the recent advances in microbiology. With the rapid widening of the scope of study relating to the microparasites, we shall soon be driven to the adoption of the French term "microbiology," rather than the more restricted German title of bacteriology.—H. L. RUSSELL.

The eight years which have elasped since the publication of the first edition of CONN'S *Agricultural bacteriology* have been years of rapid advancement in all lines of bacteriological activity. A second edition[2] finds much new material to be incorporated.

The author in his preface admits that the limitations of the term agr cultural bacteriology are puzzling. The farmer has most of the city problems of sanitation in miniature and many more in economic bacteriology. The prevention of infectious disease in man and animals; farm engineering with its problems of sanitation, water supply, and sewage disposal; the function of bacteria in the production of butter and cheese; the conservation of the soil by encouraging the growth of beneficial bacteria; the part which bacteria play in the curing and preparation of food for man and animal—these problems range over practically the whole domain of bacteriology. A text which attempts to cover such a field in a little more than three hundred pages must of necessity be an outline merely and not an exhaustive treatment of the subject. Each chapter could be easily elaborated into a volume.

In make-up the volume has improved. The pages have been reduced from 412 to 331 in number. The illustrations have been numbered and many old ones replaced by better. Part, at least, of the decrease in size is due to the elimination of the references to literature found at the end of each chapter in the first edition. The text has been entirely rewritten, though the main divisions have remained as before. The revision has resulted in increased conciseness and clearness of expression. The discussion of bacteria and the nitrogen problem is excellent for use in an agricultural high school, but seems rather inadequate as a presentation of the subject to college students with two or more years of training in chemistry

[2] CONN, H. W., Agricultural bacteriology. Second edition. pp. x+331. *figs. 64.* Philadelphia: P. Blakiston's Son and Co. 1909. $2.00.

and biology. A chapter on bacteria and soil minerals has been added. Tuberculosis is the only disease which is treated at length. The treatment of acquired immunity is misleading, in that vaccination is the only method of conferring immunity which is discussed, and the natural inference is that diphtheria and other diseases are thus treated. Antitoxins are not mentioned. A part of a chapter on fungus diseases of plants shows plainly the effect of too much condensation. The characterization of wilts, rusts, etc., on page 295 is unscientific and inaccurate. The student can gain little by a mere list of names of hosts and parasites such as this chapter contains. There is much that might be eliminated to make room for more adequate treatment of other subjects. As an introduction to the subject for the general reader or for the high-school student the volume is excellent; as a college text, however, it seems inadequate.—R. E. BUCHANAN.

NOTES FOR STUDENTS

Current taxonomic literature.—R. HÖROLD (Bot. Jahrb. **42**:251–334. 1909) presents a synoptical revision of the American Thibaudieae and carefully tabulates their geographical distribution. One monotypic genus (*Englerodoxa*) and 67 species referred to 17 genera are published as new to science.—G. MASSEE (Annals of Botany **23**:336. 1909) has published a new genus (*Gibsonia*) of the Ascomycetes; the new fungus was found growing in a drain in North Lancashire, England.—E. A. FINET (Bull. Soc. Bot. Fr. IV. **9**:97–104. *pls. 1, 2.* 1909) describes several new or noteworthy species of Orchidaceae, some of which are American.—L. A. DODE (*ibid. 232–234*) has published a new genus (*Orias*) of the Lythraceae from China.—N. L. GARDNER (Univ. Cal. Pub. Bot. **3**:371–375. *pl. 14.* 1909), under the title "New Chlorophyceae from California," has published two new monotypic genera (*Endophyton* and *Pseudodictyon*); a new species of Ulvella is also proposed.—O. TSCHOURINA (Bull. Soc. Bot. Genève II. **1**:98–101. 1909) has published a new genus (*Astrocladium*) of the Palmellaceae. The new alga was discovered in the vicinity of Geneva, Switzerland, and is represented by a single known species.—W. BIALOSUKNIA (*ibid.* 101–104) proposes a new genus (*Diplosphaera*) of the Pleurococcaceae, to which is referred but one species. The alga was isolated from the lichen, *Lecanora tartarea*, and developed as a pure culture.—G. O. MALME (*ibid.* 182) records a new species of Xyris from Brazil.—V. CALESTANI (Nuovo Giorn. Bot. Ital. N. S. **15**:355–390. 1908), under the title "Sulla classificazione delle crocifere italiane," recognizes 31 genera for Italy, including one genus (*Euxena*) published as new to science.—R. PAMPANINI (Bull. Soc. Bot. Ital. **1908**:132–134) describes a new species and variety of Tithonia indigenous to Mexico.—K. K. MACKENZIE (Muhlenbergia **5**:53–58. 1909) lists several species of Carex collected by A. A. HELLER in Nevada in 1908 and describes two new species.—P. B. KENNEDY (*ibid.* 58–61. *pl. 2*) in continuation of his "Studies in Trifolium" describes and illustrates a new species from Oregon.—W. FAWCETT and A. B. RENDLE (Journ. Botany **47**:122–129. 1909) in continuation of their studies on Jamaica orchids have published 13 new species belonging to various genera and one new genus (*Neo-urbania*); the new genus is based on

Ponera adendrobium Reichb.—J. A. PURPUS (Monats. Kateenk. **19**:52, 53, 89. 1909) describes and illustrates two new species of Cereus from Guatemala.—F. EICHLAM (*ibid.* 59, 60) has published a new variety of *Mamillaria Celsiana* Lem. from Guatemala.—T. S. BRANDEGEE (Univ. Cal. Pub. Bot. **3**:377–396. 1909), under the title "Plantae mexicanae Purpusianae," has published 43 species and one variety of angiospermous plants as new to science, and proposes the following new genera: *Setchellanthus* of the Capparidaceae, *Acanthothamnus* of the Celastraceae, and *Dichondropsis* of the Convolvulaceae.—H. D. HOUSE (Muhlenbergia **5**:65–72. 1909) has described 7 new species of American Convolvulaceae and made several new combinations.—B. SCHROEDER (Ber. Deutsch. Bot. Gesells. **27**:210–214. 1909), under the title of "Phytoplankton von Westindien," lists 71 species from West Indian waters, including one new to science.—J. BRIQUET (Ann. Conserv. et Jard. Bot. Genève **11–12**:175–193. 1908) has published 10 new species and 4 new varieties of Ranunculus and Geranium from Mexico and South America.—N. WILLE (Nyt. Mag. Naturv. **47**:— (reprint 1–21. *pls. 1–4.* 1909) describes and illustrates a new genus (*Wittrockiella*) of the Chaetophorales, represented by a single species, *W. paradoxa*. The material on which the new genus is based was collected near Lyngor on the southeast coast of Norway. The author proposes for it the new family *Wittrockiellaceae* and states that its nearest affinity is with the Chroolepidaceae.—E. HASSLER (Bull. Soc. Bot. Genève II. **1**:207–212. 1909) proposes a new genus (*Pseudobastardia*) of the Malvaceae from South America.—H. CHRIST (*ibid.* 216–236) in continuation of his treatment of the ferns of Costa Rica, for the "Primitiae florae costaricensis," has published 27 new species and one monotypic new genus (*Costaricia*), also 2 new species of Lycopodium.—E. L. GREENE (Rep. Nov. Sp. **7**:1–6. 1909) publishes 17 new species of the genus Aconitum from western America.—F. KRÄNZLIN (*ibid.* 38–41) has described new species in the Orchidaceae, some of which are from South America, and (*ibid.* 114, 115) a new species of Epidendrum from Mexico.—T. HERZOG (*ibid.* 49–69), in collaboration with different specialists, has published 37 new species of Siphonogamae from South America, chiefly from Bolivia.—A. COGNIAUX (*ibid.* 69–72) has described six new species and varieties of Orchidaceae and Melastomaceae from Paraguay, and (*ibid.* 121–123) four new orchids from Jamaica.—E. HASSLER (*ibid.* 72–78) has published six new species and varieties of Malvaceae and Leguminosae from Paraguay and proposes a new genus (*Pseudopavonia*).—A. LINGELSHEIM, F. PAX, and H. WINKLER (*ibid.* 107–114), under the title "Plantae novae bolivianae," have published 20 new species of flowering plants.—W. BECKER (*ibid.* 123, 124) records two new species of Viola from Peru.—E. PALLA (Oester. Bot. Zeits. **59**:186–194. *pl. 3.* 1909) has published several new Cyperaceae, including a new species of Bulbostylis from Bolivia.—E. ULE (Verh. Bot. Ver. Brand. **50**:69–123. 1909), in cooperation with several specialists, has published 70 new species of flowering plants from South America, based on collections made by himself in the region of the Amazon; two new genera are proposed: *Chamaeanthus* of the Commelinaceae and *Dolichodelphys* of the Rubiaceae.—P. HENNINGS (*ibid.* 129–136) has described several new fungi, including

a new monotypic genus (*Exogone*), which the author refers to the Rhizinaceae; the material on which the new genus is based was found growing on partially decayed leaves of cabbage.—T. MAKINO (Bot. Mag. Tokyo **23**:59–75. 1909) in continuation of his studies on the flora of Japan describes several new species and proposes a new monotypic genus (*Ortherodendron*) of the Celastraceae, based on *Elaeodendron japonicum* Franch. & Sav.—W. SUKATSCHEFF (Jour. Bot. St. Pétersb. **3**:124–136. 1908) gives an account of an alga recently discovered in Lake Lunoevo, Russia, for which the author proposes the generic name *Lunoevia;* illustrations supplement the description.—V. L. KOMAROV (Acta Hort. Petrop. **29**:179–362. *pls. 5–20.* 1909) presents a monographic treatment of the genus Caragana, in which 56 species are recognized, 27 being new to science; the genus has its distribution through central Asia and China.—E. B. COPELAND (Phil. Jour. Sci. **4**:1–64. *pls. 1–21.* 1909), in an article entitled "Ferns of the Malay-Asiatic region, part I," including all families of ferns for the region except Hymenophyllaceae and Polypodiaceae, recognizes 22 genera, to which are referred 196 species. The genus Cyathea dominates, being there represented by 101 species. Each genus is illustrated by reproduced photographs, but from rather fragmentary material.—C. B. ROBINSON (*ibid.* 69–105) records for the Philippine Islands three species of the Chloranthaceae, of which one is new, and some 55 species of the section Phyllanthinae of the Euphorbiaceae, of which 21 are new to science.—R. MUSCHLER (Bot. Jahrb. **43**:1–74. 1909), under the title "Sytematische und pflanzengeographische Gliederung der afrikanischen Senecio-Arten," presents a detailed consideration of the genus, as it pertains to Africa, recognizing about 500 species, 28 of which are here described for the first time.—H. D. HOUSE (Ann. N. Y. Acad. Sci. **18**:181–263. 1908) presents a monographic treatment of the North American species of Ipomoea, in which 175 species and several varieties are recognized, 30 being new. The author gives concise keys, rather full synonomy, and numerous citations of well-known series of exsiccatae, thus making a very useful synopsis for the identification of material of this group. The revision excludes Operculina, Quamoclit, Exogonium, Calonyction, Turbina, and Rivea.—P. C. STANDLEY (Muhlenbergia **5**:81–87. 1909) has described 5 new species of Castilleja from the southwest.—E. O. WOOTEN and P. C. STANDLEY (*ibid.* 87) describe a new species of Lathyrus from New Mexico.—J. M. GREENMAN.

Cytology of a Drosera hybrid.—Since the preliminary announcement of ROSENBERG'S work on the hybrid *Drosera longifolia*×*rotundifolia*, cytologists have awaited with some impatience the more detailed account, which has now appeared.[3] Besides giving a comparative study of the external features of the two parents and their hybrid, the present paper describes the behavior of the chromosomes in critical phases of the life-history of both parents, and gives an extended account of the chromosomes of the hybrid.

[3] ROSENBERG, O., Cytologische und morphologische Studien über *Drosera longifolia*×*rotundifolia*. Kungl. Svenska Vetenskapsakad. Handl. **43**:1–64. *pls. 1–4.* 1909.

In *D. longifolia* there are 40 chromosomes in the nuclei of the sporophyte and 20 in those of the gametophyte, while in *D. rotundifolia* the numbers are 20 and 10 respectively. The chromosomes of *D. rotundifolia* are somewhat smaller, as well as less numerous. The behavior of the chromatin in a hybrid between two such forms is naturally of some importance.

In the hybrid, called *D. obovata*, the nuclei of the sporophyte show regularly 30 chromosomes, the anticipated number, but in the nuclei of spore mother cells the condition is unique. At the metaphase of the heterotypic mitosis in the pollen mother cell there appear 10 double chromosomes, presumably resulting from the pairing of 10 chromosomes of *D. longifolia* with the 10 of *D. rotundifolia*. Besides, there are 10 smaller single chromosomes, presumably belonging to *D. longifolia*. These 10 smaller chromosomes are irregularly distributed; some enter the daughter nucleus at the close of this mitosis, while others remain in the cytoplasm and may organize small nuclei, as in the well-known case of Hemerocallis. The behavior at the second mitosis is similar. The four spores of the pollen tetrad stick together, so that it is possible to determine the entire number of chromosomes in the four nuclei. Counting the chromosomes in the four nuclei and including those of the dwarf nuclei, the number is about 60. In any given spore the number ranges from 10 to 15, with 14 the most frequent. In a preliminary paper, ROSENBERG concluded that two of the spores of a tetrad belonged to *D. longifolia* and two to *D. rotundifolia*. This conclusion is now withdrawn, and differences in the size of spores is attributed to differences in the number of chromosomes. Sometimes a generative cell is formed, but usually the contents of the spore begin to disorganize before this stage is reached. At the time of shedding, the pollen grain has a normal exine, but the contents are usually dead.

In the formation of four megaspores from the mother cell the behavior is very similar to that just described. Occasionally, there is a well-developed embryo sac, but in most cases disorganization begins before the four-nucleate stage is reached.

ROSENBERG crossed the hybrid *D. obovata* with *D. longifolia*, and while usually there was no result, he obtained a few embryos. These contained at least 33 chromosomes, and in one case 37 were counted. The theoretical number would be 35.

The principal conclusions are (1) that the chromosome is an individual organ of the cell, and (2) reduction of chromosomes is brought about by a fusion of the chromosomes of the two parents.—CHARLES J. CHAMBERLAIN.

Mildew on Alchemilla species.—STEINER[4] has published an interesting paper on the specialization of *Sphaerotheca Humuli* (DC.) Burr on various species of Alchemilla. In addition to finding that the mildew on Alchemilla is confined to species of this genus, he also claims to be able to distinguish "minor biological species" within this genus of host plants. For example, conidia

[4] STEINER, J. A., Die Spezialization der Alchemillen-bewohnenden *Sphaerotheca Humuli* (DC.) Burr. Centralbl. f. Bakt. etc. **21**²:677–726. 1908.

from *A. pastoralis* and *A. flexicaulis* are alike in infecting capacity, except that conidia from the former will only partially infect *A. pubescens*, and not *A. alpigena* at all; while conidia from *A. flexicaulis* partially infect *A. alpigena*, *A. pubescens* being entirely immune. Another case is that of the mildew on *A. impexa* which does not infect *A. alpina vera* or *A. nitida*, while conidia from *A. pastoralis* partially infect these hosts. Otherwise the two mildews are alike. STEINER further found that conidia from species of the *Vulgares* group will not produce full infection on alpine species, although conidia from alpine species produce full infection on the *Vulgares* species. STEINER supposes that the mildew on the alpine species came originally from *Vulgares* species and is only partially adapted to the new hosts. He also believes that the appearance of the mildew on alpine species is due to unfavorable environment.

STEINER also claims to have found "bridging species;" for example, conidia from *A. nitida* infect *A. impexa*, but not *A. fallax*, while conidia from *A. impexa* will infect *A. fallax*. Thus the mildew is carried over from *A. nitida* to *A. fallax* through *A. impexa*. Similarly, *A. pastoralis* and *A. impexa* transfer the mildew from *A. connivens* and *A. pubescens* to *A. micans*. In addition to the fact that only a few tests were made, STEINER does not tell us what are the infecting powers of the mildews produced in this way on *A. micans* and *A. fallax*.

His conclusions would be more convincing if based on a larger number of tests. A large number of foreign infections also occurred in his experiments, no less than 71 foreign infections occurring in a total of 380 tests. The results are presented very clearly by means of a series of well-devised diagrams.—GEORGE M. REED.

Cytology of Florideae.—Cytological studies on the Florideae have been comparatively rare, partly on account of the difficulty in securing material, but principally on account of the difficult technic. Quite recently KURSSANOW has published[5] the results of his studies on three different forms of red algae: *Helminthora divaricata*, *Nemalion lubricum*, and *Helminthocladia purpurea*. His investigations did not deal with nuclear details, but rather with the morphology of fertilization of the carpogonium, the development of carpospores, and the structure of the chromatophores.

He failed to find a nucleus in the trichogyne of Nemalion and Helminthora; the trichogyne in these forms seems to be an extension of the carpogonium. He believes that such a condition is found only in the simplest forms of red algae, and agrees with the reviewer that a trichogyne with a nucleus, and yet without a partition wall between it and a carpogonium, as in Polysiphonia, may be a forerunner of the multicellular trichogyne found in the Laboulbeniaceae. The spermatium (sperm) has a single nucleus, agreeing with the reviewer's description of Polysiphonia. He thinks that a uninuclear condition in the sperm may perhaps be universal in red algae. In Nemalion, contrary to WOLFE'S results, the chromatophore has, in its center, a well-formed pyrenoid which is composed

[5] KURSSANOW, L., Beiträge zur Cytologie der Florideen. Flora **99**:311–336. *pls. 2, 3.* 1909.

of two parts, a central portion and the surrounding zone. The pyrenoid is influenced by its environment, and easily becomes swollen and dissolved, leaving vacuoles in its place. Such a compound structure of the pyrenoid is shown only in the stained preparation, and when it is not differentiated with stains the pyrenoid appears quite homogeneous. SCHMITZ's description of the pyrenoid as a homogeneous body may perhaps be based upon the unstained material.—SHIGÉO YAMANOUCHI.

Karyokinesis in Oedogonium.—Since STRASBURGER's and KLEBAHN's work on Oedogonium, there had been little published on mitosis in this form until WISSELINGH's paper appeared. STRASBURGER's material was *O. tumidulum* Kg., KLEBAHN's *O. Boscii* Witte, and WISSELINGH's material was *O. cyathigerum* Witte,[6] fixed in Flemming's solution. After being left in the solution for one day, it was treated with 20 per cent. chromic acid. By the action of the Flemming solution and the chromic acid solution, the cell wall and cell contents become entirely dissolved, and the nuclear membrane is also dissolved by the action of 20 per cent. chromic acid solution. The chromosomes during mitosis were studied in their isolated condition.

The chief points of interest are as follows: The mitosis in Oedogonium agrees with that of higher plants; the development of chromosomes out of the nuclear network, the formation of the nuclear plate, the longitudinal splitting of the chromosomes, the reconstruction of daughter nuclei seem like these processes in Fritillaria and Leucojum, two forms which were also studied by VON WISSELINGH. In Oedogonium, the chromosomes, 19 in number, and differing greatly from one another in length, are connected by fine fibrils. The nucleolus does not take part in forming chromosomes, but disappears at the beginning of mitosis, and there appear in daughter nuclei new nucleoli, which later unite into one.—SHIGÉO YAMANOUCHI.

Mycorhiza.—PEKLO announces in a preliminary paper[7] the results of his studies on the epiphytic mycorhiza of Carpinus and Fagus, with brief reference also to the endophytes of *Alnus glutinosa* and *Myrica Gale*.

In Carpinus, as a reaction to the penetration of the tissues of the young rootlet, tannins increase (as the author has also determined for Monotropa[8]), and this restricts the fungus to the intercellular spaces. Nourishing itself partly on this glucoside and other foods in the cortex, the fungus forms the jacket, the outermost hyphae of which often die. Isolation of the fungus was finally accomplished by using a decoction of old thick mycorhizas, which proved very specific for the

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[6] WISSELINGH, C. VON, Ueber die Karyokinese bei Oedogonium. Beih. Bot. Centralbl. **23**:139–156. *pl. 7*. 1908.

[7] PEKLO, J., Beiträge zur Lösung des Mykorhiza-Problems. Ber. Deutsch. Bot. Gesells. **27**:239–247. 1909.

[8] ———, Die epiphytischen Mykorhizen nach neuen Untersuchungen. I. *Monotropa Hypopitys* L. Bull. Böhm. Akad. Wiss. 00:000. 1908.

infecting fungus. In this the inner hyphae began to grow and broke through the outer layers, and on this mycelium, whose origin was clear, conidiophores and conidia arose within three days. These showed it to be a Penicillium (Citromyces) very like *P. geophilum*, and similar results were reached with Fagus. Fungi of this group were also found in the forest soil where mycorhiza of Fagus was abundant. Carpinus was not available for experiments on reinfection, but a considerable number of young roots of a two-year-old Fagus showed infection from pure cultures of the Carpinus mycorhiza, as well as from several other species of forest Penicillia.—C. R. B.

Respiration.—For about a dozen plants Mme. MAIGE has determined the amount of O_2 fixed and CO_2 evolved by the stamens and pistils as compared with an equal weight of leaf tissue, both in air and in pure hydrogen.[9] She finds both aerobic and anaerobic respiration, tested thus, to be much (2–18 times) more active in the floral organs than in the leaf; and, with one exception, more vigorous in the pistil than in the stamen, and in the anther than in the filament. These results confirm the early ones (1822) of DE SAUSSURE, as to the relative rate of respiration of the floral organs and the leaves; but DE SAUSSURE found stamens more active than pistils. For the conciseness of this paper Mme. MAIGE is much to be commended.

JENSEN[10] finds that the alcoholic fermentation of sugar proceeds by two stages and he therefore predicates two enzymes, glucose being split by dextrase (glucase?) into dioxyacetone and this by "dioxyacetonase" into CO_2 and alcohol. But in respiration, with oxidase and free oxygen present, the dioxyacetone, produced as in fermentation, breaks up into CO_2 and water, the main end-products of aerobic respiration.—C. R. B.

Transpiration.—SAMPSON and ALLEN, declaring that too little account has been taken of the effect of physical factors on transpiration, furnish further data on this subject.[11] Comparing evaporation from equal areas in equal times they find that there is little variation for plants of the same species under the same conditions of development and exposure; that of the same species the sun form evaporates 2–4 times as much as the shade form, whether the two are tested in the sun or shade, a difference which they ascribe chiefly to the greater number of stomata in the sun form (20–60 per cent.); that the increased evaporation with altitude, *caeteris paribus*, is due to lower pressure and not to differences in light or humidity; that generally acid solutions accelerate and alkaline solutions retard evaporation, but without relation to concentration; that evaporation is greater

[9] MAIGE, MME. G., Recherches sur la respiration de l'étamine et du pistil. Rev. Gén. Bot. **21**:32–38. 1909.

[10] JENSEN, P. BOYSEN, Die Zersetzung des Zuckers während des Respirationsprozesses. Ber. Deutsch. Bot. Gesells. **26a**:666, 667. 1908.

[11] SAMPSON, A. W., AND ALLEN, LOUISE M. Influence of physical factors on transpiration. Minn. Bot. Stud. **4**:33–59. 1909.

from plants in coarse than in fine soils; and that a "bog xerophyte," *Scirpus lacustris*, loses about twice as much water as *Helianthus annuus*, on account of its loose structure, the air spaces being estimated at 80 per cent. of the total volume and the internal surface as 15 times the external.—C. R. B.

Anthocyan.—On the vexed question of the formation of anthocyan, COMBES furnishes[12] first a very clear and compact summary of the previous researches. He then demonstrated that the close relations between the accumulation of carbohydrates and the formation of anthocyan, pointed out by the researches of OVERTON and MOLLIARD on artificially nourished plants, exist also in nature, however the pigmentation is provoked. The insoluble carbohydrates behave differently, according to the occasion of the pigmentation; but the sugars, glucosides, and dextrins behave alike in all cases, the two former varying in amount directly as the anthocyan, the dextrins diminishing as the sugars and glucosides increase. The insoluble carbohydrates, consequently, appear not to share directly in the formation of the red pigment. COMBES concludes that the anthocyans, which are probably cyclic glucosides, are formed at the expense of neither preexistent sugars and glucosides nor chromogens, but arise at the same time as other glucosides, as part of the general accumulation of such bodies.—C. R. B.

Chlorophyll bodies.—Morphological distinctions between chlorophyll bodies, found in a great number and variety of plants, have been pointed out by D'ARBAUMONT,[13] who divides them into two categories, chloroplasts and pseudochloroplasts. The former, held to be morphologically superior, seem to include the bodies usually recognized under that name, without admixture of the latter, from which they are distinguished by not swelling in water (at least *in situ*), and by not being stained, with rare exceptions, by acid aniline blue. The pseudochloroplasts, on the contrary, usually swell in water and become vividly colored in the stain. They are of four types, all small, more or less varied in shape, with different degrees of green coloration, and variously intermixed. The members of the two categories are formed in the same way, either with or without the cooperation of starch,[14] and both, without reference to their mode of origin, may or may not form starch.—C. R. B.

Morphology of Symplocarpus.—In an investigation of *Symplocarpus foetidus*, ROSENDAHL[15] has obtained the following results: the primordia of the flowers

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[12] COMBES, R., Rapports entre les composés hydrocarbonés et la formation de l'anthocyane. Ann. Sci. Nat. Bot. IX. **9**: 275–303. 1909.

[13] D'ARBAUMONT, J., Nouvelle contribution a l'étude des corps chlorophylliens. Ann. Sci. Nat. Bot. IX. **9**: 197–229. 1909.

[14] *Cf.* BELZUNG, E., Nouvelles recherches sur l'origine des grains d'amidon et des grains de chlorophylle. Ann. Sci. Nat. Bot. VII. **13**: 17. 1891; Jour. de Bot. **9**: 67, 102. 1895.

[15] ROSENDAHL, C. OTTO, Embryo sac development and embryology of *Symplocarpus foetidus*. Minn. Bot. Studies **4**¹: 1–9. *pls. 1–3*. 1909.

appear 18–20 months before the pollination period, and the ovules are formed late during the season preceding pollination; the single archesporial cell produces four distinct megaspores; an antipodal tissue of a considerable number of cells with large nuclei is developed; endosperm formation begins with free nuclear division, and this is followed by a walled-tissue which fills the embryo sac and encroaches upon the integuments and the chalaza; a filamentous proembryo (2 or 4 cells) becomes club-shaped to ovoid, and a short suspensor of several rows of cells is differentiated from the usual monocotyledonous embryo; in its growth the embryo completely destroys the endosperm and all other ovular structures, and comes to lie naked in the cavity of the ovary, so that there are no seeds in the ordinary sense.—J. M. C.

Morphology of Caulophyllum.—The seed and seedling of *Caulophyllum thalictroides* have been studied by BUTTERS,[16] with the following results: the fleshy testa incloses a very hard endosperm, which has almost completely destroyed the inner integument; the proembryo is massive and pear-shaped and the cotyledons appear late; the first season's growth after germination is usually entirely subterranean, the cotyledons together forming an effective haustorium; the first leaves are usually scalelike and inclose a winter bud; each cotyledon sends three vascular bundles into the hypocotyl, which finally form a tetrarch root; secondary thickening takes place in the hypocotyl, resulting in the formation of a continuous zone of xylem about the pith.—J. M. C.

Temperature and locomotion.—TEODORESCO reports[17] movements in certain organisms at temperatures far lower than have heretofore been recorded. Thus he found zoospores of Dunaliella motile down to temperatures of −17° to −22°.5 C., and others at −5° to −12°.7 C. The limits vary with species and even with individuals. There seems to be much more activity in winter, even among freshwater organisms, than has been supposed.—C. R. B.

Carbon monoxid.—KRASCHÉNNIKOFF, after a careful series of experiments, reports[18] that CO cannot be used by green plants to form carbohydrate. The view of BOTTOMLEY AND JACKSON,[19] which was really not adequately supported by their experiments, the only ones interpreted in favor of such use, is distinctly negatived.—C. R. B.

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[16] BUTTERS, FREDERIC K., The seeds and seedling of *Caulophyllum thalictroides*. Minn. Bot. Studies **4**:11–32. *pls. 4–10.* 1909.

[17] TEODORESCO, E. C., Recherches sur les mouvements de locomotion der organismes inférieurs aux basses températures. Ann. Sci. Nat. Bot. IX. **9**:231–274. 1909.

[18] KRASCHÉNNIKOFF T., La plante verte assimile-t-elle l'oxyde de carbone? Rev. Gén. Bot. **21**:177–193. *pl. 10.* 1909.

[19] Proc. Roy. Soc. Lond. **72**:130–131. 1903.